(19)日本国特許庁（JP） (12)公開特許公報（A） (11)特許出願公開番号
特開2001-352986
(P2001-352986A)
(43)公開日 平成13年12月25日(2001.12.25)

(51)Int.Cl.'	識別記号	FI		テーマコード'(参考)
C12N 15/09	ZNA	A01H 5/00	A	2B030
A01H 5/00		A01K 67/027		2G045
A01K 67/027		67/033	501	4B024
67/033	501	A61K 39/395	D	4B063
A61K 38/00			N	4B064

審査請求 未請求 請求項の数56 OL （全 52 頁） 最終頁に続く

(21)出願番号 特願2000-175475(P2000-175475)

(22)出願日 平成12年6月12日(2000.6.12)

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(54)【発明の名称】 新規ポリペプチド

(57)【要約】
【課題】NF-κBの活性化が関与する疾患の治療薬、予防薬および診断薬の探索、開発に有用なポリペプチド、該ポリペプチドをコードするDNA、該DNAのアンチセンスDNA／RNA、該DNAを用いた遺伝子治療、該ポリペプチドを認識する抗体、該ポリペプチドの活性上昇改変体、該ポリペプチドのドミナントネガティブ変異体、およびこれらの利用法を提供する。
【解決手段】NF-κBを活性化するポリペプチドを同定し、該ポリペプチドをコードするDNA、および該ポリペプチドを認識する抗体を製造する。これらはNF-κBの活性化が関与する疾患の治療薬の探索ならびに診断に利用することができる。

【特許請求の範囲】
【請求項1】 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれるアミノ酸配列を有するポリペプチド。
【請求項2】 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれるアミノ酸配列において1以上のアミノ酸が欠失、置換および／または付加されたアミノ酸配列からなり、かつNF-κBの活性を上昇させる活性を有するポリペプチド。
【請求項3】 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれるアミノ酸配列と60％以上の相同性を有するアミノ酸配列を含み、かつNF-κBの活性を上昇させる活性を有するポリペプチド。
【請求項4】 請求項1～3のいずれか1項に記載のポリペプチドをコードするDNA。
【請求項5】 配列番号6～10のいずれかで表される塩基配列を有するDNA。
【請求項6】 請求項4または5に記載のDNAとストリンジェントな条件下でハイブリダイズするDNAであり、かつ転写因子NF-κBの活性を上昇させる活性を有するポリペプチドをコードするDNA 。
【請求項7】 請求項4～6のいずれか1項に記載のDNAをベクターに組み込んで得られる組換え体ベクター。
【請求項8】 請求項4～6のいずれか1項に記載のDNAと相同な配列からなるRNAをベクターに組み込んで得られる組換え体ベクター。
【請求項9】 RNAが1本鎖である請求項8記載の組換え体ベクター。
【請求項10】 請求項7記載の組換え体ベクターを保有する形質転換体。
【請求項11】 形質転換体が、微生物、動物細胞、植物細胞、および昆虫細胞からなる群より選ばれる形質転換体である、請求項10記載の形質転換体。
【請求項12】 微生物が、Escherichia属に属する微生物である、請求項11記載の形質転換体。
【請求項13】 動物細胞が、マウス・ミエローマ細胞、ラット・ミエローマ細胞、マウス・ハイブリドーマ細胞、CHO細胞、BHK細胞、アフリカミドリザル腎臓細胞、Namalwa細胞、Namalwa KJM-1細胞、ヒト胎児腎臓細胞およびヒト白血病細胞から選ばれる動物細胞である、請求項11記載の形質転換体。
【請求項14】 昆虫細胞が、Spodoptera frugiperdaの卵巣細胞、Trichoplusia niの卵巣細胞およびカイコの卵巣細胞から選ばれる昆虫細胞である、請求項11記載の形質転換体。
【請求項15】 形質転換体が、非ヒトトランスジェニック動物またはトランスジェニック植物である、請求項10記載の形質転換体。
【請求項16】 請求項10～14のいずれか1項に記載の形質転換体を培地に培養し、培養物中に請求項1～3のいずれか1項に記載のポリペプチドを生成、蓄積させ，該培養物から該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造方法。
【請求項17】 請求項7記載の組換え体DNAを保有する非ヒトトランスジェニック動物を飼育し、請求項1～3のいずれか1項に記載のポリペプチドを該動物中に生成、蓄積させ、該動物中より該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造方法。
【請求項18】 蓄積が動物のミルク中であることを特徴とする、請求項17記載の製造法。
【請求項19】 請求項7記載の組換え体DNAを保有するトランスジェニック植物を栽培し、請求項1～3のいずれか1項に記載のポリペプチドを該植物中に生成、蓄積させ、該植物中より該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造法。
【請求項20】 請求項4～6のいずれか1項に記載のDNAを用い、in vitroでの転写・翻訳系により、該DNAのコードするポリペプチドを合成することを特徴とする、該ポリペプチドの製造法。
【請求項21】 請求項1～3のいずれか1項に記載のポリペプチドを認識する抗体。
【請求項22】 請求項4～6のいずれか1項に記載のDNAの塩基配列中の連続した5～60塩基からなる配列を有するオリゴヌクレオチドまたは該ヌクレオチドと相補的な配列を有するオリゴヌクレオチド。
【請求項23】 請求項4～6のいずれか1項に記載のDNAまたは請求項22記載のオリゴヌクレオチドをプローブとして用いてハイブリダイゼーションを行うことを含む、請求項1～3のいずれか1項に記載のポリペプチドをコードするDNAの発現を検出する方法。
【請求項24】 請求項22記載のオリゴヌクレオチドをプライマーとして用いたポリメラーゼ・チェイン・リアクションを行うことを含む、請求項1～3のいずれか1項に記載のポリペプチドをコードするDNAの発現を検出する方法。
【請求項25】 請求項4～6のいずれか1項に記載のDNAまたは請求項22記載のオリゴヌクレオチドを用い、ハイブリダイゼーション法により、請求項1～3のいずれか1項に記載のポリペプチドをコードするDNAの変異を検出する方法。
【請求項26】 請求項22記載のオリゴヌクレオチドを用いたポリメラーゼ・チェイン・リアクションを行うことを含む、請求項1～3のいずれか1項に記載のポリペプチドをコードするDNAの変異を検出する方法。
【請求項27】 感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓

β細胞の障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、または異常な細胞増殖を伴う疾患を検出するために用いる、請求項２３～２６のいずれか１項に記載の方法。

【請求項２８】　感染や炎症を伴う疾患が、微生物感染、ＨＩＶ感染、慢性Ｂ型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、エンドトキシンショック、敗血症、移植片対宿主疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、膵臓β細胞の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍である、請求項２７記載の方法。

【請求項２９】　請求項４～６のいずれか１項に記載のＤＮＡまたは請求項２２記載のオリゴヌクレオチドを用いることを特徴とする、請求項１～３のいずれか１項に記載のポリペプチドをコードするＤＮＡの転写またはｍＲＮＡの翻訳を抑制する方法。

【請求項３０】　請求項４～６のいずれか１項に記載のＤＮＡまたは請求項２２記載のオリゴヌクレオチドを用いることを特徴とする、請求項１～３のいずれか１項に記載のポリペプチドをコードするＤＮＡのプロモーター領域および転写制御領域を取得する方法。

【請求項３１】　請求項１～３のいずれか１項に記載のポリペプチドを含む医薬。

【請求項３２】　請求項４～６のいずれか１項に記載のＤＮＡ、または請求項８若しくは９のいずれか１項に記載の組換え体ベクターを含む医薬。

【請求項３３】　請求項２１記載の抗体を含む医薬。

【請求項３４】　請求項２２記載のオリゴヌクレオチドを含む医薬。

【請求項３５】　ポリペプチドが免疫賦活作用を有することを特徴とする請求項３１記載の医薬。

【請求項３６】　免疫賦活作用を介して抗腫瘍活性および抗ウイルス活性を誘導することを特徴とする請求項３５記載の医薬。

【請求項３７】　医薬が、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓β細胞の障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、異常な細胞増殖を伴う疾患または神経細胞の障害に基づく疾患の治療および／または予防のための医薬である、請求項３２～３４のいずれか１項に記載の医薬。

【請求項３８】　医薬が、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓細胞の障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、または異常な細胞増殖を伴う疾患の診断のための医薬である、請求項３２～３４のいずれか１項に記載の医薬。

【請求項３９】　感染や炎症を伴う疾患が、微生物感染、ＨＩＶ感染、慢性Ｂ型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、エンドトキシンショック、敗血症、移植片対宿主疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、膵臓β細胞の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍であり、神経細胞の障害に基づく疾患がアルツハイマー病または虚血性脳疾患である、請求項３７または３８記載の医薬。

【請求項４０】　請求項１～３のいずれか１項に記載のポリペプチドを用いることを特徴とする、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓β細胞障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、または異常な細胞増殖を伴う疾患または神経細胞の障害に基づく疾患の治療および／または予防のための医薬のスクリーニング方法。

【請求項４１】　感染や炎症を伴う疾患が、微生物感染、ＨＩＶ感染、慢性Ｂ型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、エンドトキシンショック、敗血症、移植片対宿主疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、膵臓β細胞の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍であり、神経細胞の障害に基づく疾患がアルツハイマー病または虚血

性脳疾患である、請求項４０記載の医薬のスクリーニング法。

【請求項４２】　請求項４０または４１記載のスクリーニング方法により得られる、請求項１～３のいずれか１項に記載のポリペプチドに特異的に作用する医薬。

【請求項４３】　請求項３０記載の方法により得られる請求項１～３のいずれか１項に記載のポリペプチドをコードするＤＮＡのプロモーター領域および転写制御領域を用いることを特徴とする、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓β細胞障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、異常な細胞増殖を伴う疾患または神経細胞の障害に基づく疾患の治療および／または予防のための医薬のスクリーニング方法。

【請求項４４】　感染や炎症を伴う疾患が、微生物感染、ＨＩＶ感染、慢性Ｂ型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、エンドトキシンショック、敗血症、移植片対宿主疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、膵臓β細胞の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍であり、神経細胞の障害に基づく疾患がアルツハイマー病または虚血性脳疾患である、請求項４３記載の医薬のスクリーニング方法。

【請求項４５】　請求項４３または４４記載のスクリーニング方法により得られる、請求項１～３のいずれか１項に記載のポリペプチドをコードするＤＮＡのプロモーター領域および転写制御領域に特異的に作用する医薬。

【請求項４６】　請求項２１記載の抗体を用いることを特徴とする、請求項１～３のいずれか１項に記載のポリペプチドの免疫学的検出法。

【請求項４７】　請求項２１記載の抗体を用いて、請求項１～３のいずれか１項に記載のポリペプチドを検出することを特徴とする免疫組織染色法。

【請求項４８】　請求項２１記載の抗体を用いることを特徴とする、請求項１～３のいずれか１項に記載のポリペプチドをコードするＤＮＡの転写もしくは翻訳を抑制または促進する物質をスクリーニングする方法。

【請求項４９】　請求項１～３のいずれか１項に記載のポリペプチドをコードするＤＮＡの発現が一部または完全に抑制されているノックアウト非ヒト動物。

【請求項５０】　請求項１～３のいずれか１項に記載のポリペプチドの有する活性が一部または完全に抑制されているノックアウト非ヒト動物。

【請求項５１】　請求項１～３のいずれか１項に記載のポリペプチドを用いることを特徴とする、請求項１～３のいずれか１項に記載のポリペプチドのＮＦ－κＢ活性化に対してドミナントネガティブ活性を有する変異体ポリペプチドのスクリーニング方法。

【請求項５２】　請求項５１記載のスクリーニング方法により得られる、請求項１～３のいずれか１項に記載のポリペプチドのＮＦ－κＢ活性化に対してドミナントネガティブ活性を有する変異体ポリペプチド。

【請求項５３】　請求項５２記載の変異体ポリペプチドをコードするＤＮＡ。

【請求項５４】　請求項１～３のいずれか１項に記載のポリペプチドを用いることを特徴とする、請求項１～３のいずれか１項に記載のポリペプチドのＮＦ－κＢ活性化に対して該活性化を上昇させる変異を有する変異体ポリペプチドのスクリーニング方法。

【請求項５５】　請求項５４記載のスクリーニング方法により取得される、請求項１～３のいずれか１項に記載のポリペプチドのＮＦ－κＢ活性化能が上昇した変異体ポリペプチド。

【請求項５６】　請求項５５記載の変異体ポリペプチドをコードするＤＮＡ。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、新規なポリペプチド、該ポリペプチドをコードするＤＮＡ、該ＤＮＡをベクターに組み込んで得られる組換え体ＤＮＡ、該組換え体ＤＮＡを保有する形質転換体、該形質転換体を利用した該ポリペプチドの製造法、該ＤＮＡより得られるオリゴヌクレオチドを用いた該ＤＮＡの発現量と変異の解析法、該ポリペプチドを認識する抗体、該抗体を用いた免疫組織染色法、該ポリペプチドに欠失・挿入・置換等により変異を導入した活性上昇改変体、該ポリペプチドに欠失・挿入・置換等により変異を導入したドミナントネガティブ変異体、該ポリペプチドの活性を変動させる化合物のスクリーニング法、該ＤＮＡの発現を変動させる化合物のスクリーニング法、該ＤＮＡの転写を司るプロモーターＤＮＡ、該プロモーターＤＮＡによる転写の効率を変動させる化合物のスクリーニング法、これらのスクリーニング法により得られる化合物、および該ＤＮＡを欠損または変異させたノックアウト動物等に関する。

【０００２】

【従来の技術】nuclear factor-kappaB（以下、ＮＦ－κＢ）は、１９８６年にＢ細胞における免疫グロブリン軽鎖（Ig light chain）遺伝子の発現にかかわるエンハンサーに結合する転写因子として同定された（Cell, 4

6, 705-716 (1986)、Cell, 47,921-928 (1986)]。

【0003】NF-κBは、Relファミリーに属する複数の分子のヘテロダイマーで構成されており、多くの細胞で一般に誘導されてくるNF-κBは、p50とRelAのヘテロダイマーと考えられる〔Mol. Cell. Biol., 12, 674-684 (1992)]。NF-κBを制御する因子IκBの存在も明らかとなっており、IκBは、無刺激時にはNF-κBと複合体を形成しており、NF-κBの核移行シグナルをマスクすることにより、核移行を抑制している〔Science, 242, 540-546 (1988)、Cell, 65, 1281-1289 (1991)、Cell, 68, 1109-1120 (1992)、EMBO J., 12,3893-3901 (1993)、Cell, 78, 773-785 (1994)、Cell, 87, 13-20 (1996)]。腫瘍壊死因子α（以下、TNF-α）等で細胞を刺激すると、IκBは後述するシグナル伝達分子により32および36番目のセリンがリン酸化、続いてユビキチン化され、プロテアソームによって分解される。IκBが分解されると、NF-κBは核への移行が可能となり、エンハンサーを持った様々な遺伝子発現を誘導するようになる〔Cell,80, 529-532 (1995)、Cell, 80, 57 3-582 (1995)]。

【0004】NF-κBを活性化する物質あるいは刺激として、サイトカイン〔TNF-α、腫瘍壊死因子β（以下、TNF-β）、インターロイキン1α（以下、IL-1α）、インターロイキン1β（以下、IL-1β）、インターロイキン2（以下、IL-2）、白血病阻止因子（以下、LIF）等）、T細胞マイトジェン（抗原刺激、レクチン、抗T細胞レセプター抗体、抗CD2抗体、抗CD3抗体、抗CD28抗体、Caイオノフォア）、B細胞マイトジェン（抗IgM抗体、anti-CD40）、ロイコトリエン、リポ多糖（以下、LPS）、ホルボールミリステートアセテート（以下、PMA）、寄生体感染、ウイルス感染〔ヒト免疫不全症ウイルス（以下、HIV-1）、ヒトT細胞白血病ウイルス1（以下、HTLV-1）、B型肝炎ウイルス（以下、HBV）、エプスタインーバールウイルス（以下、EBV）、サイトメガロウイルス（以下、CMV）、単純ヘルペスウイルス1（以下、HSV-1）、ヒトヘルペスウイルス6（以下、HHV-6）、ニューカッスル病ウイルス（以下、NDV）、センダイウイルス、アデノウイルス等）、ウイルス産物（二本鎖RNA、Tax、HBX、EBNA-2、LMP-1等）、DNA破壊物質類、タンパク質合成インヒビター類（例えばシクロヘキシミド）、紫外線、放射線、酸化ストレス等が知られている〔Biochemica et Biophysica Acta, 1072, 63-80 (1991)、Annu. Rev. Cell Biol. 10, 405-455 (1994)]。

【0005】また、NF-κBの活性化により誘導発現される分子としては、（1）炎症反応・免疫応答に関る分子群、（2）アポトーシスの抑制に関る分子群、（3）発生・分化に関る分子群、（4）ウイルスに関する分子群等が知られており〔Biochemica et Biophysica Acta, 1072, 63-80 (199 1)、Annu. Rev. Cell Biol. 10, 405-455 (1994)]、誘導発現は多岐にわたっている。

【0006】誘導発現される分子として、具体的には、サイトカイン〔IL-1α、IL-1β、IL-2、インターロイキン3（以下、IL-3）、インターロイキン6（以下、IL-6）、インターロイキン8（以下、IL-8）、インターロイキン12（以下、IL-12）、TNF-α、TNF-β、インターフェロンβ（以下、IFN-β）]、細胞増殖因子〔マクロファージコロニー刺激因子（以下、M-CSF）、顆粒球マクロファージコロニー刺激因子（以下、GM-CSF）、顆粒球コロニー刺激因子（以下、G-CSF）]、レセプター〔インターロイキン1レセプター（以下、IL-1R）アンタゴニスト、インターロイキン2レセプターα（以下、IL-2Rα）、免疫グロブリンκ軽鎖（以下、Ig-κ-LC）、T細胞レセプターβ、主要組織適合抗原（以下、MHC）クラスI, II, β2-ミクログロブリン）、接着因子〔endothelial leucocyte adhesionmolecule-1(以下、ELAM-1)、vascular cell adhesion molecule-1(以下、VCAM-1)、intercellular adhesion molecule-1(以下、ICAM-1)]、急性期タンパク質（血清アミロイドA前駆タンパク質、アンギオテンシノーゲン、補体因子B、補体因子C3、補体因子C4）、誘導型NO合成酵素（以下、iNOS）、シクロオキシゲナーゼ2（以下、COX-2）、血管内皮細胞成長因子受容体（以下、VEGF-R2）、転写因子〔c-Rel、p105、Iκ-α、c-Myc、インターフェロン調節因子（以下、IRF-1）]、ビメンチン、ウイルス〔HIV-1、HIV-2、アカゲザル免疫不全症ウイルス（以下、SIVmac）、CMV、HSV-1、アカゲザルウイルス40（以下、SV40）、アデノウイルス〕等が知られている〔蛋白質核酸酵素, 41, 1198-1209 (1996)]。

【0007】NF-κB活性化に関するシグナル伝達は、TNF-αおよびIL-1について解明が進んでいる。TNF-αからの活性化シグナルにおいては、TNFレセプター（TNFR1またはTNFR2）、TNF receptor-associate d death domain protein(以下、TRADD)、TNFR-associated factor-2 (以下、TRAF2)、receptor interacting protein (以下、RIP)、NF-κB-inducing kinase (以下、NIK)、IκB kinase (以下、IKK)〔IKKα、IKKβ、IKKγ（NEMO）]、IKK-complex-associated protein (以下、IKAP) 等が活性化分子として見出されている。〔EMBO J., 14, 2876-288 3 (1995)、Science, 267, 1485-1489 (1995)、GENES & DEVELOPMENT, 9, 1586-1597(1995)、Cell, 84, 853-862 (1996)、Nature, 388, 548-554 (1997)、Cell, 90,373-383 (1997)、Science, 278, 860-866 (1 997)、Science, 278, 866-869 (1997)、Cell, 91, 243-252 (1997)、Nature, 395, 292-296 (1998)]。

【0008】IL-1からの活性化シグナルにおいては、IL-1 recptor 1 (以下、IL-1R1)、IL-1 receptor accessory protein (以下、IL-1RAcP)、Myd88、IL-1 receptor-associated kinase (以下、IRAK) TNF receptor-associated factor 6 (以下、TRAF6)、TAK1 binding protein 1 (以下、TAB1)、Transforming growth factor-β-activated kinase 1(TAK1)等が活性化分子として見出されている〔Science, 270, 2008-2011 (1995)、Nature, 398, 252-256 (1999)]。

【0009】一方、NF-κBをリン酸化する酵素（NF-κBキナーゼ）がNF-κBシグナルの増強に関わっているとも考えられてきた〔J. Biol. Chem. 268, 26790-26795 (1993)、EMBO J. 13, 4597-4607 (1994)]。以上のように、NF-κBの活性化には非常に多くの分子が関与していることは知られているが、同定された全ての分子の役割が解明されているわけではない。紫外線や酸化ストレス等のTNF-αやIL-1以外の刺激では、NF-κBの活性化に関わる分子は、ほとんど解明されていないのが実状である。さらに、Relファミリー分子の組織特異的発現を見ても、組織特異的なNF-κBの活性化機構が予想される〔Science, 284, 313-316 (1999)、Science, 284, 316-320 (1999)、Science, 284, 321-325 (1999)、Immunity, 10, 421-429 (1999)、Nature Genet, 22, 74-77 (1999)]。

【0010】以上より、NF-κBの活性化に関わっている未知の分子は、生体内にまだ多く存在すると考えられ、これらの遺伝子を発見し利用することは、病態の解明あるいはNF-κBが関与する疾患の治療にとって、大変有用である。前述したNF-κBを活性化する分子群あるいはNF-κBの活性化によって誘導発現する分子群からもわかるように、NF-κBは生体内の免疫応答の昂進において非常に重要な役割を担っている。抗腫瘍あるいは抗ウイルス活性を有するTNF-αやIL-1等のサイトカインは、その作用の主要部分をNF-κBの活性化を通して発揮している。また、NF-κBにより誘導発現するIL-1, IL-2, IL-12, TNF-α, IFN-β等のサイトカインも、生体や組織における免疫反応を昂進し、抗腫瘍あるいは抗ウイルス活性を有している。

【0011】このように、実際の疾患においてNF-κBの活性化が、腫瘍やウイルスを抑制することは周知の事実であり、生体内あるいは生体の一部組織においてNF-κBの活性を人為的に上昇させることは、免疫応答の昂進あるいは抗腫瘍・抗ウイルス活性の増強において非常に効果があると考えられる。従って、NF-κBを活性化するポリペプチドおよびそれをコードするDNAの発見および取得、さらにはNF-κB活性化上昇変異体の発見および取得は、抗腫瘍・抗ウイルスをターゲットとした医薬において大変有用である。

【0012】一方で、NF-κBによって誘導発現するIL-1、IL-6、IL-8、TNF-α等のサイトカインは、炎症性サイトカインとも呼ばれ、これらのサイトカインによって過度に昂進された免疫応答が各種疾患の原因ともなっている。これらのサイトカインは、マクロファージ、好中球、リンパ球等を活性化し、炎症組織において増悪の方向に働く。また、NF-κBにより誘導されたELAM-1、VCAM-1、ICAM-1等の接着分子は、白血球の組織への浸潤を促進し、炎症組織での白血球の集積を昂進する〔Mol. Cell. Biol., 14, 5701 (1994)、Mol. Cell. Biol., 14, 5820 (1994),、Pro. Natl. Acad. Sci USA, 90, 3943 (1993)]。iNOSやCOX-2等の酵素は、それぞれ一酸化窒素（以下、NO）やプロスタグランディンE2を産生し、急性炎症や血管の拡張に作用する。

【0013】すなわち、NF-κBは、これらの細胞あるいは分子を介して、急性炎症および慢性炎症において中心的役割を担っていると考えられる。実際に、慢性関節リウマチの滑膜、クローン病の腸管、喘息の肺組織等では、NF-κBの活性化が報告されている。したがって、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、慢性B型肝炎、慢性C型肝炎、移植片対宿主疾患、インスリン依存性・非依存性糖尿病、外傷性脳損傷、炎症性腸疾患、敗血症、微生物感染等、炎症が関与する疾患全般において、NF-κBは、病態解明および治療薬開発の重要なターゲットである。

【0014】癌との関連では、バーキットリンパ腫（Burkitt lymphoma）、ホジキン（Hodgkin）病、T, B, NK細胞リンパ腫、EBV関連胃癌等が、EBVが原因とされる。特にEBVがコードするlatent membrane protein（以下、LMP1）は、TRADDやTRAFと結合が可能で、宿主のNF-κBを活性化し、不死化に関与していると考えられる〔EMBO J., 16, 6478-6485 (1997)、J. Virology,69, 2168-2174 (1995)、Oncogene, 18, 7161-7167 (1999)、Gene Therapy, 5,905-912 (1998)]。また、成人T細胞白血病（adult T-cell leukemia: ATL）は、HTLV-1による感染が原因であり、特にHTLV-1がコードするTaxが、IκBへの結合あるいはIKKの活性化を通じて、NF-κBを活性化し、アポトーシスを阻害していると考えられる〔J. Biol. Chem., 273, 15891-15894 (1999)、J. Biol. Chem., 274, 34417-34424 (1999)]。NF-κBが誘導する各種接着分子は、癌細胞の転移に関与しているし、NF-κBによるアポトーシス阻害活性やVEGF-R2を介した血管新生は、癌細胞の増殖に関与している。以上のように、NF-κBは、癌の分野においても重要な創薬あるいは治療ターゲットである。

【００１５】さらに、エイズ等、癌以外のＮＦ－κＢを転写因子として含むウイルス性疾患においても、ＮＦ－κＢは重要な創薬あるいは治療ターゲットである。また、虚血性脳疾患等の虚血再還流障害もＮＦ-κＢ活性化による細胞浸潤、アポトーシスの抑制等が原因という報告があり、動脈硬化、再狭窄等も含め、平滑筋細胞の異常な分化増殖を伴う疾患の発症にＮＦ-κＢが重要な役割を果たしていると考えられる。

【００１６】近年ステロイドの抗炎症作用やアスピリンの抗炎症作用等がＮＦ－κＢの阻害によるものであることが明らかにされてきたが（Scelence, 270, 283-286 (1995)、Scelence, 270, 286-290 (1995)、Nolecular and Cellular Biology, 15, 943-953 (1995)）、ＮＦ－κＢを特異的に阻害するものとしてスクリーニングされた薬剤はない。既存のＮＦ－κＢの阻害に関わるものとして知られてきた薬剤は副作用が強いことや選択性・特異性が低い等、問題点も多く、強力かつ副作用の少ない新しい抗炎症薬の開発を目的として、ＮＦ－κＢをターゲットにした化合物探索が行われている。以上より、ＮＦ－κＢを活性化する新規ポリペプチドは産業上有用であり、これらポリペプチドおよびそれをコードするＤＮＡの取得が求められてきた。

【００１７】

【発明が解決しようとする課題】本発明は、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エンドトキシンショック、敗血症、微生物感染、慢性Ｂ型肝炎、慢性Ｃ型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、外傷性脳損傷、炎症性腸疾患等の感染や炎症を伴う疾患、バーキットリンパ腫、ホジキン病、各種リンパ腫、成人Ｔ細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、関節リウマチ、変形性関節炎等の異常な線維芽細胞や滑膜組織の活性化を伴う疾患、エイズ等のウイルス性疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄等の平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群（ＳＩＲＳ：systemic inflammatory response syndrome）、成人呼吸窮迫症候群（ＡＲＤＳ：adult respiratory distress syndrome）等の治療薬、予防薬および診断薬の探索、開発に有用なポリペプチド、該ポリペプチドをコードするＤＮＡ、該ＤＮＡのアンチセンスＤＮＡ／ＲＮＡ、該ＤＮＡを用いた遺伝子治療、該ポリペプチドを認識する抗体、該ポリペプチドの活性上昇改変体、該ポリペプチドのドミナントネガティブ変異体、およびこれらの利用法を提供することにある。

【００１８】

【課題を解決するための手段】本発明者らは、上記課題を解決するべく鋭意検討を行った結果、新規なアミノ酸配列を含むＮＦ-κＢの活性化を促す因子および該因子をコードするＤＮＡを取得することに成功し、本発明を完成させるに至った。即ち、本発明は以下の（１）～（５４）に関する。

【００１９】（１）　配列番号１～５のいずれかで表されるアミノ酸配列からなる群より選ばれるアミノ酸配列を有するポリペプチド。

（２）　配列番号１～５のいずれかで表されるアミノ酸配列からなる群より選ばれるアミノ酸配列において１以上のアミノ酸が欠失、置換および／または付加されたアミノ酸配列からなり、かつＮＦ－κＢの活性を上昇させる活性を有するポリペプチド。

【００２０】（３）　配列番号１～５のいずれかで表されるアミノ酸配列からなる群より選ばれるアミノ酸配列と６０％以上の相同性を有するアミノ酸配列を含み、かつＮＦ－κＢの活性を上昇させる活性を有するポリペプチド。

（４）　（１）～（３）のいずれか一項に記載のポリペプチドをコードするＤＮＡ。

（５）　配列番号６～１０のいずれかで表される塩基配列を有するＤＮＡ。

【００２１】（６）　（４）または（５）に記載のＤＮＡとストリンジェントな条件下でハイブリダイズするＤＮＡであり、かつ転写因子ＮＦ－κＢの活性を上昇させる活性を有するポリペプチドをコードするＤＮＡ。

（７）　（４）～（６）のいずれか１項に記載のＤＮＡをベクターに組み込んで得られる組換え体ベクター。

（８）　（４）～（６）のいずれか１項に記載のＤＮＡと相同な配列からなるＲＮＡをベクターに組み込んで得られる組換え体ベクター。

【００２２】（９）　ＲＮＡが１本鎖である（８）記載の組換え体ベクター。

（１０）　（７）記載の組換え体ベクターを保有する形質転換体。

（１１）　形質転換体が、微生物、動物細胞、植物細胞、および昆虫細胞からなる群より選ばれる形質転換体である、（１０）記載の形質転換体。

（１２）　微生物が、Escherichia属に属する微生物である、（１１）記載の形質転換体。

【００２３】（１３）　動物細胞が、マウス・ミエローマ細胞、ラット・ミエローマ細胞、マウス・ハイブリドーマ細胞、ＣＨＯ細胞、ＢＨＫ細胞、アフリカミドリザル腎臓細胞、Ｎａｍａｌｗａ細胞、Ｎａｍａｌｗａ ＫＪＭ－１細胞、ヒト胎児腎臓細胞およびヒト白血病細胞から選ばれる動物細胞である、（１１）記載の形質転換体。

（１４）　昆虫細胞が、Spodoptera frugiperdaの卵巣細胞、Trichoplusia niの卵巣細胞およびカイコの卵巣細胞から選ばれる昆虫細胞である、（１１）記載の形質転換体。

【００２４】（１５）　形質転換体が、非ヒトトランスジェニック動物またはトランスジェニック植物である、（１０）記載の形質転換体。

（１６）　（１０）～（１４）のいずれか１項に記載の形質転換体を培地に培養し、培養物中に（１）～（３）のいずれか１項に記載のポリペプチドを生成、蓄積させ、該培養物から該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造方法。

【００２５】（１７）　（７）記載の組換え体ＤＮＡを保有する非ヒトトランスジェニック動物を飼育し、

（１）～（３）のいずれか１項に記載のポリペプチドを該動物中に生成、蓄積させ、該動物中より該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造方法。

（１８）　蓄積が動物のミルク中であることを特徴とする、（１７）記載の製造法。

【００２６】（１９）　（７）記載の組換え体ＤＮＡを保有するトランスジェニック植物を栽培し、（１）～（３）のいずれか１項に記載のポリペプチドを該植物中に生成、蓄積させ、該植物中より該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造法。

（２０）　（４）～（６）のいずれか１項に記載のＤＮＡを用い、in vitroでの転写・翻訳系により、該ＤＮＡのコードするポリペプチドを合成することを特徴とする、該ポリペプチドの製造法。

【００２７】（２１）　（１）～（３）のいずれか１項に記載のポリペプチドを認識する抗体。

（２２）　（４）～（６）のいずれか１項に記載のＤＮＡの塩基配列中の連続した５～６０塩基からなる配列を有するオリゴヌクレオチドまたは該ヌクレオチドと相補的な配列を有するオリゴヌクレオチド。

（２３）　（４）～（６）のいずれか１項に記載のＤＮＡまたは（２２）記載のオリゴヌクレオチドをプローブとして用いてハイブリダイゼーションを行うことを含む、（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡの発現を検出する方法。

【００２８】（２４）　（２２）記載のオリゴヌクレオチドをプライマーとして用いたポリメラーゼ・チェイン・リアクションを行うことを含む、（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡの発現を検出する方法。

（２５）　（４）～（６）のいずれか１項に記載のＤＮＡまたは（２２）記載のオリゴヌクレオチドを用い、ハイブリダイゼーション法により、（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡの変異を検出する方法。

【００２９】（２６）　（２２）記載のオリゴヌクレオチドを用い、ポリメラーゼ・チェイン・リアクションを行うことを含む、（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡの変異を検出する方法。

（２７）　感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓β細胞の障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、または異常な細胞増殖を伴う疾患を検出するために用いる、（２３）～（２６）のいずれか１項に記載の方法。

【００３０】（２８）　感染や炎症を伴う疾患が、微生物感染、ＨＩＶ感染、慢性Ｂ型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、エンドトキシンショック、敗血症、移植片対宿主疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、膵臓β細胞の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍である、（２７）記載の方法。

【００３１】（２９）　（４）～（６）のいずれか１項に記載のＤＮＡまたは（２２）記載のオリゴヌクレオチドを用いることを特徴とする、（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡの転写またはｍＲＮＡの翻訳を抑制する方法。

（３０）　（４）～（６）のいずれか１項に記載のＤＮＡまたは（２２）記載のオリゴヌクレオチドを用いることを特徴とする、（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡのプロモーター領域および転写制御領域を取得する方法。

【００３２】（３１）　（１）～（３）のいずれか１項に記載のポリペプチドを含む、医薬。

（３２）　（４）～（６）のいずれか１項に記載のＤＮＡ、または（８）若しくは（９）のいずれか１項に記載の組換え体ベクターを含む医薬。

（３３）　（２１）記載の抗体を含む医薬。

（３４）　（２２）記載のオリゴヌクレオチドを含む医薬。

【００３３】（３５）　ポリペプチドが免疫賦活作用を有することを特徴とする（３１）記載の医薬。

（３６）　免疫賦活作用を介して抗腫瘍活性および抗ウイルス活性を誘導することを特徴とする（３５）記載の医薬。

（３７）　医薬が、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性

化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓β細胞の障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、異常な細胞増殖を伴う疾患または神経細胞の障害に基づく疾患の治療および／または予防のための医薬である、（３２）～（３４）のいずれか１項に記載の医薬。

【００３４】（３８）　医薬が、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓細胞の障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、または異常な細胞増殖を伴う疾患の診断のための医薬である、（３２）～（３４）のいずれか１項に記載の医薬。

【００３５】（３９）　感染や炎症を伴う疾患が、微生物感染、ＨＩＶ感染、慢性Ｂ型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、エンドトキシンショック、敗血症、移植片対宿主疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、膵臓β細胞の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍であり、神経細胞の障害に基づく疾患がアルツハイマー病または虚血性脳疾患である、（３７）または（３８）記載の医薬。

【００３６】（４０）　（１）～（３）のいずれか１項に記載のポリペプチドを用いることを特徴とする、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓β細胞障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、異常な細胞増殖を伴う疾患または神経細胞の障害に基づく疾患の治療および／または予防のための医薬のスクリーニング方法。

【００３７】（４１）　感染や炎症を伴う疾患が、微生物感染、ＨＩＶ感染、慢性Ｂ型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、エンドトキシンショック、敗血症、移植片対宿主疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、膵臓β細胞の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍であり、神経細胞の障害に基づく疾患がアルツハイマー病または虚血性脳疾患である、（４３）記載の医薬のスクリーニング方法。

【００３８】（４２）　（４０）または（４１）記載のスクリーニング方法により得られる、（１）～（３）のいずれか１項に記載のポリペプチドに特異的に作用する医薬。

（４３）　（３０）記載の方法により得られる（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡのプロモーター領域および転写制御領域を用いることを特徴とする、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、膵臓β細胞障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、異常な細胞増殖を伴う疾患または神経細胞の障害に基づく疾患の治療および／または予防のための医薬のスクリーニング方法。

【００３９】（４４）　感染や炎症を伴う疾患が、微生物感染、ＨＩＶ感染、慢性Ｂ型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、エンドトキシンショック、敗血症、移植片対宿主疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、膵臓β細胞の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍であり、神経細胞の障害に基づく疾患がアルツハイマー病または虚血性脳疾患である、（４３）記載の医薬のスクリーニング方法。

【００４０】（４５）　（４３）または（４４）記載のスクリーニング方法により得られる、（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡのプロモーター領域および転写制御領域に特異的に作用する医薬。

（４６）　（２１）記載の抗体を用いることを特徴とする、（１）～（３）のいずれか１項に記載のポリペプチドの免疫学的検出法。

（４７）　（２１）記載の抗体を用いて、（１）～（３）のいずれか１項に記載のポリペプチドを検出することを特徴とする免疫組織染色法。

【００４１】（４８）　（２１）記載の抗体を用いることを特徴とする、（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡの転写もしくは翻訳を抑制または促進する物質をスクリーニングする方法。

（４９）　（１）～（３）のいずれか１項に記載のポリペプチドをコードするＤＮＡの発現が一部または完全に抑制されているノックアウト非ヒト動物。

（５０）　（１）～（３）のいずれか１項に記載のポリペプチドの有する活性が一部または完全に抑制されているノックアウト非ヒト動物。

【００４２】（５１）　（１）～（３）のいずれか１項に記載のポリペプチドを用いることを特徴とする、

（１）～（３）のいずれか１項に記載のポリペプチドのＮＦ－κＢ活性化に対してドミナントネガティブ活性を有する変異体ポリペプチドのスクリーニング方法。

（５２）　（５１）記載のスクリーニング方法により取得られる、（１）～（３）のいずれか１項に記載のポリペプチドのＮＦ－κＢ活性化に対してドミナントネガティブ活性を有する変異体ポリペプチド。

（５３）　（５２）記載の変異体ポリペプチドをコードするＤＮＡ。

【００４３】（５４）　（１）～（３）のいずれか１項に記載のポリペプチドを用いることを特徴とする、

（１）～（３）のいずれか１項に記載のポリペプチドのＮＦ－κＢ活性化に対して酸活性化を上昇させる変異を有する変異体ポリペプチドのスクリーニング方法。

（５５）　（５４）記載のスクリーニング方法により取得される、（１）～（３）のいずれか１項に記載のポリペプチドのＮＦ－κＢ活性化能が上昇した変異体ポリペプチド。

（５６）　（５５）記載の変異体ポリペプチドをコードするＤＮＡ。

【００４４】

【発明の実施の形態】本発明のポリペプチドとしては、

１.配列番号１～５のいずれかで表されるアミノ酸配列からなる群より選ばれるアミノ酸配列を有するポリペプチド

２.配列番号１～５で表されるアミノ酸配列からなる群より選ばれるアミノ酸配列において１以上のアミノ酸が欠失、置換および／または付加されたアミノ酸配列からなり、かつＮＦ－κＢの活性を上昇させる活性を有するポリペプチド

３.配列番号１～５のいずれかで表されるアミノ酸配列からなる群より選ばれるアミノ酸配列と６０％以上の相同性を有するアミノ酸配列を含み、かつＮＦ－κＢの活性を上昇させる活性を有するポリペプチドを挙げることができる。

【００４５】上記のアミノ酸配列を有するポリペプチドにおいて１以上のアミノ酸が欠失、置換および／または付加されたアミノ酸配列を有するポリペプチドは、Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press,1989（以下、モレキュラー・クローニング第２版と略す）、Current Protocols in Molecular Biology, John Wiley & Sons, 1987-1997（以下、カレント・プロトコールズ・イン・モレキュラー・バイオロジーと略す）、Nucleic Acids Research , 10, 6487, （1982）、Proc. Natl. Acad. Sci., USA, 79, 6409 （1982）、Gene, 34, 315 （1985）、Nucleic Acids Research, 13, 4431 （1985）、Proc. Natl. Acad. Sc i USA, 82, 488 （1985）等に記載の部位特異的変異導入法を用いて、例えば配列番号１～５のいずれかのアミノ酸配列を有するポリペプチドをコードするＤＮＡに部位特異的変異を導入することにより行うことができる。欠失、置換および／または付加されるアミノ酸の数は１から数個であり、その数は特に限定されないが、上記の部位特異的変異導入法等の周知の技術により、欠失、置換もしくは付加できる程度の数であり、例えば、１～数十個、好ましくは１～２０個、より好ましくは１～１０個、さらに好ましくは１～５個である。

【００４６】また、本発明のポリペプチドとしては、配列番号１～５のいずれかに記載のアミノ酸配列と６０％以上の相同性を有するアミノ酸配列を含む。配列番号１～５のいずれかに記載のアミノ酸配列との相同性は、ＢＬＡＳＴ〔J. Mol . Biol.,215. 403 (1990) 〕やＦＡＳＴＡ（Methods in Enzymology, 183, 63-69）等の解析ソフトで、デフォルト（初期設定）のパラメータを用いて計算したときに、少なくとも６０％以上、好ましくは７０％以上、より好ましくは８０％以上、さらに好ましくは９０％以上、特に好ましくは９５％以上、最も好ましくは９７％以上が好ましい。

【００４７】本発明のＤＮＡとしては、

１.請求項１～３のいずれか一項に記載のポリペプチドをコードするＤＮＡ

２.請求項４記載のＤＮＡとストリンジェントな条件下でハイブリダイズするＤＮＡであり、かつ転写因子ＮＦ－κＢの活性を上昇させる活性を有するポリペプチドをコードするＤＮＡ

３.配列番号６～１０のいずれかで表される塩基配列を有するＤＮＡを挙げることができる。

【００４８】一般に１つのアミノ酸に対して複数種の遺伝暗号が存在するため、配列番号６～１０のいずれかとは異なる塩基配列を有するＤＮＡであっても本発明のポリペプチドをコードしていれば本発明のＤＮＡに含まれる。ストリンジェントな条件下でハイブリダイズするＤＮＡとは、例えば配列番号６、７、８、９または１０で表される塩基配列を有するＤＮＡ等の本発明のＤＮＡま

たはその一部の断片をプローブとして、コロニー・ハイブリダイゼーション法、プラーク・ハイブリダイゼーション法あるいはサザンブロットハイブリダイゼーション法等を用いることにより得られるDNAを意味し、具体的には、コロニーあるいはプラーク由来のDNAを固定化したフィルターを用いて、0．7～1．0mol／lの塩化ナトリウム存在下、65℃でハイブリダイゼーションを行った後、0．1～2倍濃度のSSC溶液（1倍濃度のSSC溶液は、150mmol／l塩化ナトリウム，15 mmol／lクエン酸ナトリウムよりなる）を用い、65℃条件下でフィルターを洗浄することにより同定できるDNAを挙げることができる。ハイブリダイゼーションは、モレキュラー・クローニング第2版、カレント・プロトコールズ・イン・モレキュラー・バイオロジー、D NACloning 1: Core Techniques, A Practical Approach, Second Edition, Oxford University, 1995等に記載されている方法に準じて行うことができる。

【0049】ハイブリダイズ可能なDNAとして具体的には、BLASTやFASTA等の解析ソフトで、デフォルト（初期設定）のパラメータを用いて計算したときに、配列番号6、7、8、9または10で表される塩基配列と少なくとも60％以上の相同性を有するDNA、好ましくは70％以上、より好ましくは80％以上、さらに好ましくは90％以上、特に好ましくは95％以上、最も好ましくは98％以上の相同性を有するDNAを挙げることができる。

【0050】以下、本発明を詳細に説明する。

1．本発明のDNAの調製

ヒトmRNAは、市販のもの（例えば、Clontech社製）を用いてもよいし、以下のごとくヒト組織から調製してもよい。組織から全RNAを調製する方法としては、チオシアン酸グアニジンートリフルオロ酢酸セシウム法（Methods in Enzymology, 154, 3 (1987)）、酸性チオシアン酸グアニジン・フェノール・クロロフォルム（AGPC）法（Analytical Biochemistry, 162, 156 (1987)、実験医学，9．1937 (1991)）等が挙げられる。また、全RNAからpolyA+ RNAとしてmRNAを調製する方法としては、オリゴ（dT）固定化セルロースカラム法（モレキュラー・クローニング第2版）等が挙げられる。さらに、FastTrack mRNA Isolation Kit（Invitrogen社製）、Quick Prep mRNA Purification Kit（Pharmacia社製）等のキットを用いることによりmRNAを調製できる。

【0051】調製したヒト組織mRNAからcDNAライブラリーを作製する。cDNAライブラリー作製法としては、モレキュラー・クローニング第2版、カレント・プロトコールズ・イン・モレキュラー・バイオロジー、A Laboratory Manual, 2 nd Ed., 1989等に記載された方法、あるいは市販のキット、例えばSuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning（Life Technologies社製）、ZAP-cDNA Synthesis Kit（STRATAGENE社製）を用いる方法等が挙げられる。

【0052】cDNAライブラリーを作製するためのクローニングベクターとしては、大腸菌K12株中で自立複製できるものであれば、ファージベクター、プラスミドベクター等いずれでも使用できる。具体的には、ZAP Express（STRATAGENE社製、Strategies, 5．58，(1992)）、pBluescript II SK(+)（Nucleic Acids Research, 17．9494 (1989)）、Lambda ZAP II（STRATAGENE社製）、λgt10、λgt11（DNA cloni ng, A Practical Approach, 1．49 (1985)）、λTriplEx（Clontech社製）、λExCell（Pharmacia社製）、pT7T318U（Pharmacia社製）、pcD2（Mol. Cell. Biol., 3．280 (1983)）およびpUC18（Gene, 33，103 (1985)）等を挙げることができる。

【0053】宿主微生物としては、大腸菌に属する微生物であればいずれでも用いることができる。具体的には、Escherichia coli XL1-Blue MRF'（STRATAGENE社製、Strategies, 5．81 (1992)）、Escherichia coli C600（Genetics, 39．440 (1954)）、Esherichia coli Y1088（Science, 222，778 (1983)）、Escherichiacoli Y1090（Science, 222．778 (1983)）、Escherichia coli NM522（J. Mol. Biol., 166．1 (1983)）、Kshe richia coli K802（J. Mol. Biol., 16．118 (1966)）、Escherichia coli JM105（Gene, 38．275 (1985)）等が用いられる。

【0054】このcDNAライブラリーを、そのまま以下の解析に用いてもよいが、不完全長cDNAの割合を下げ、なるべく完全長cDNAを効率よく取得するために、菅野らが開発したオリゴキャップ法（Gene, 138．171, (1994)、Gene, 200，149 (1997)、蛋白質核酸酵素，41，603 (1996)、実験医学，11，2491 (1993)、cDNAクローニング，羊土社 (1996)、遺伝子ライブラリーの作製法，羊土社 (1994)）を用いて調製したcDNAライブラリーを以下の解析に用いてもよい。

【0055】作製したcDNAライブラリーから各クローンを単離し、それぞれのクローンについてcDNAの塩基配列を末端から、通常用いられる塩基配列解析方法、例えばサンガー（Sanger）らのジデオキシ法（Proc. Natl. Acad. Sci. USA, 74.54 63 (1977)）あるいはABI PRISM377DNAシークエンサー（PE Biosystem社製）等の塩基配列分析装置を用いて分析することにより、該DNAの塩基配列を決定する。得られた塩基配列をアミノ酸配列に翻訳することにより、このDNAがコードするポリペプチドのアミノ酸配列を得ることができる。

【0056】また、得られた塩基配列をGenBank、EMBL等の塩基配列データベース中の塩基配列とBLAST、FASTA等の相同性解析プログラムを用いて比較することにより、得られた塩基配列が新規な塩基配列かどうか、また得られた塩基配列と相同性をもつ塩基配列を検索することができる。また塩基配列より得られたアミノ酸配列をSwissProt、PIR、GenPept等のアミノ酸配列データベースと比較することにより、その塩基配列がコードするポリペプチドと相同性をもつポリペプチド、例えばラットとは別の生物種での相当する遺伝子に由来するポリペプチドや同じような活性や機能をもつと推定されるファミリータンパク質を検索することができる。

【0057】データベース検索で明らかになった相同遺伝子の塩基配列を基に、該遺伝子に特異的なプライマーを設計し、上記のようにして取得した一本鎖cDNAまたはcDNAライブラリーを鋳型としてPCRを行う。増幅断片が得られた際には、該断片を適当なプラスミドにサブクローニングする。サブクローニングは、増幅断片をそのまま、あるいは制限酵素やDNAポリメラーゼで処理後、定法によりベクターに組込むことにより行うことができる。ベクターとしては、pBluescript SK(-)（Stratagene社製）、pBluescript II SK(+)（Stratagene社製）、pDIRECT（Nucreic Acid s Research, 18，6069 (1990)）、pCR-Script Amp SK(+)（Stratagene社製）、pT7Blue（Novagen社製）、pCRII（Invitrogen社製）、pCR-TRAP（Genehunter社製）、pNo TA77（5'→3' 社製）等を挙げることができる。

【0058】配列番号6～10のいずれかの塩基配列からなるDNAが一旦取得され、その塩基配列が決定された後は、該塩基配列の5'端および3'端の塩基配列に基づいたプライマーを調製し、ヒトまたは非ヒト動物の組織または細胞に含まれるmRNAから合成したcDNAあるいはcDNAライブラリーを用いてDNAの増幅を行うことにより、本発明のDNAを取得することができる。

【0059】また、配列番号6～10のいずれかの塩基配列よりなるDNAの全長あるいは一部をプローブとして、ヒトまたは非ヒト動物の組織または細胞に含まれるmRNAから合成したcDNAあるいはcDNAライブラリーに対してコロニーハイブリダイゼーションやプラークハイブリダイゼーション（モレキュラー・クローニング第2版）を行うことにより、本発明のDNAを取得することができる。

【0060】決定されたDNAの塩基配列に基づいて、ホスフォアミダイト法を利用したパーキン・エルマー社のDNA合成機（model 392）等のDNA合成機で化学合成することにより、本発明のDNAを取得することもできる。本発明のオリゴヌクレオチドとしては、オリゴDNA、オリゴRNA等のオリゴヌクレオチド、および該オリゴヌクレオチドの誘導体（以下、誘導体オリゴヌクレオチド）等が挙げられる。

【0061】該オリゴヌクレオチドまたは該オリゴヌクレオチドと相補的な配列に相当するオリゴヌクレオチド（以下、アンチセンスオリゴヌクレオチド）として、例えば、検出したいmRNAの一部の塩基配列において、5'末端側の塩基配列に相当するセンスプライマー、3'末端側の塩基配列に相当するアンチセンスプライマー等を挙げることができる。ただし、mRNAにおいてウラシルに相当する塩基は、オリゴヌクレオチドプライマーにおいてはチミジンとなる。

【0062】センスプライマーおよびアンチセンスプライマーとしては、両者の融解温度（Tm）および塩基数が極端に変わることのないオリゴヌクレオチドで、5～60塩基、好ましくは10～50塩基数のものが挙げられる。誘導体オリゴヌクレオチドとしては、オリゴヌクレオチド中のリン酸ジエステル結合がホスフォロチオエート結合に交換されたもの、オリゴヌクレオチド中のリン酸ジエステル結合がN3'－P5'ホスフォアミデート結合に交換されたもの、オリゴヌクレオチド中のリボースとリン酸ジエステル結合がペプチド核酸結合に交換されたもの、オリゴヌクレオチド中のウラシルがC－5プロピニルウラシルで置換されたもの、オリゴヌクレオチド中のウラシルがC－5チアゾールウラシルで置換されたもの、オリゴヌクレオチド中のシトシンがC－5プロピニルシトシンで置換されたもの、オリゴヌクレオチド中のシトシンがフェノキサジン修飾シトシン（phenoxazine-modified cytosine）で置換されたもの、オリゴヌクレオチド中のリボースが2'-メトキシエトキシリボースで置換されたもの等が挙げられる（細胞工学，16，1463 (1997)）。

【0063】2．本発明のDNAのNF－κB活性化の検出法

（1）活性検出に用いる宿主細胞

本発明において、DNAの活性を検出するために用いる宿主細胞としては、DNAを細胞内に導入できる細胞ならいかなる細胞も用いることができる。該細胞として、例えば、細菌・古細菌、藻類、菌類、植物、動物等に由来した細胞が挙げられる。具体的には、下記生物由来の細胞が挙げられる。

【0064】細菌・古細菌としてはEscherichia coliやBacillus subtilis等が挙げられる。藻類としてはSynechococcus属やSynechocystis属の藍藻等が挙げられる。植物としてはタバコ、アラビドプシス、トマト、ジャガイモ、ナタネ、ワタ、ダイズ、イネまたはトウモロコシ等が挙げられる。菌類としてはSaccharomyces cerevisiaeやAspergillus nigar等が挙げられる。動物としては哺乳動物、節足動物等が挙げられる。

【0065】哺乳動物としてはヒト、サル、マウス、ラット、モルモットまたはミンク等が挙げられる。具体的には、ヒトの細胞としてはT細胞株Jurkat（アメリカン・タイプ・カルチャー・コレクション（以下、ATCCと略記する）の番号TIB-512の細胞株）、B細胞株N

ａｍａｌｗａ（ＡＴＣＣ ＣＲＬ-1432）、子宮癌細胞株ＨｅＬａ（ＡＴＣＣ ＣＣＬ-2）、腎細胞株２９３（Ｊ. Ｇｅｎ. Ｖｉｏｌ. 36, 59-72 (1977)）等を用いることができる。ヒト以外の哺乳動物の細胞としては、サル腎細胞株ＣＯＳ－１（ＡＴＣＣ ＣＲＬ-16 50）、サル腎細胞株ＣＯＳ－７（ＡＴＣＣ ＣＲＬ-1651）、チャイニーズ・ハムスター卵巣（Ｃｈｉｎｅａｓｅ Ｈａｍｓｔｅｒ Ｏｖａｒｙ）細胞株ＣＨＯ（ＡＴＣＣ ＣＲＬ-9096、ＡＴＣＣ ＣＣＬ-61）、マウス細胞株Ｂａ／Ｆ３（ＲＩＫＥＮ Ｃｅｌｌ Ｂａｎｋ ＲＣＢ0805）、マウス細胞株Ｌ９２９（ＲＩＫＥＮ Ｃｅｌｌ Ｂａｎｋ ＲＣＢ0081）、ラット細胞株ＮＲＫ－４９Ｆ（ＡＴＣＣ ＣＲＬ-1570）、ミンク細胞株Ｍｖ１Ｌｕ（ＡＴＣＣ ＣＣＬ-64）等を用いることができる。節足動物としては、カイコが挙げられる。具体的には、Ｓｐｏｄｏｐｔｅｒａ ｆｒｕｇｉｐｅｒｄａ Ｓｆ９株やＳｆ２１株等を用いることができる。治療用タンパク性医薬品や医薬品のスクリーニングターゲットとなるＤＮＡの探索が目的の場合は、哺乳動物の細胞、特にヒトの細胞を宿主とすることが好ましい。

【００６６】（２）宿主細胞への遺伝子導入法

本発明のＤＮＡを宿主細胞に導入する方法としては、宿主細胞に遺伝子を導入する方法であればどのような方法でも用いることができる。例えば、エレクトロポレーション法（羊土社 バイオマニュアルシリーズ４，23）、リン酸カルシウム法（羊土社 バイオマニュアルシリーズ４，13）、ＤＥＡＥデキストラン法（羊土社 バイオマニュアルシリーズ４，16）、リポフェクション法（羊土社 バイオマニュアルシリーズ４，28）、マイクロインジェクション法（羊土社 バイオマニュアルシリーズ４，36）、アデノウイルス法（羊土社 バイオマニュアルシリーズ４，43）、ワクシニアウイルス法（羊土社 バイオマニュアルシリーズ４，59）レトロウイルスベクター法（羊土社 バイオマニュアルシリーズ４，74）等の公知の方法を用いることができる。

【００６７】（３）本発明のＤＮＡを取得する方法

本発明のＤＮＡは、細胞で発現させることによりＮＦ－κＢを活性化できるため、細胞におけるＮＦ－κＢの活性化を検出することが可能な方法を用いることにより本発明のＤＮＡを取得することができる。ＮＦ－κＢの活性化を検出する方法として、以下の方法が挙げられる。

【００６８】例えば、細胞抽出液を用いる方法として、転写制御領域への結合をゲルシフト法（羊土社 バイオマニュアルシリーズ５，107）等により解析する方法、ＩκＢのリン酸化やユビキチン化をウエスタンブロット法（羊土社 バイオマニュアルシリーズ７，179）等により検出する方法が挙げられる。また、さらに効率よく検出する方法として、レポーター遺伝子を用いて検出する方法を挙げることができる。レポーター遺伝子としては、ルシフェラーゼ、ヒト胎盤アルカリ・ホスファターゼ、β－ガラクトシダーゼ、ウロキナーゼ、クロラムフェニコールアセチルトランスフェラーゼ、ヒト成長ホルモン、各種Ｇｒｅｅｎｆｌｕｏｒｅｓｃｅｎｔ ｐｒｏｔｅｉｎ（以下、ＧＦＰ）

等をコードする遺伝子を用いることができる。レポーター遺伝子に連結するプロモーターとしては、ＮＦ－κＢにより転写されうるプロモーターであるならいかなるプロモーターも用いることができる。例えば、ＮＦ－κＢの活性化により発現が制御されている遺伝子のプロモーター領域を染色体ＤＮＡから制限酵素消化によって切り出すことにより単離したプロモーターＤＮＡ断片、染色体ＤＮＡを鋳型としてＰＣＲ法によって増幅することによって得られるプロモーターＤＮＡ断片、または該プロモーターの塩基配列を有する合成ＤＮＡ断片等が挙げられる。

【００６９】具体的には、ＩＬ－１α、ＩＬ－１β、ＩＬ－２、ＩＬ－３、ＩＬ－６、ＩＬ－８、ＩＬ－１２、ＴＮＦ－α、ＴＮＦ－β、ＩＦＮ－β、Ｍ－ＣＳＦ、ＧＭ－ＣＳＦ、Ｇ－ＣＳＦ、Ｌ－２Ｒα、Ｉｇ－κ－ＬＣ、Ｔ細胞レセプターβ、ＭＨＣクラスＩ、β2-ミクログロブリン、ＬＡＭ－１、ＶＣＡＭ－１、ＩＣＡＭ－１、血清アミロイドＡ前駆タンパク質、アンギオテンシノーゲン、補体因子Ｂ、補体因子Ｃ3、補体因子Ｃ4、、ｉＮＯＳ、ＣＯＸ－２、ＶＥＧＦ－Ｒ２、ｃ－Ｒｅｌ、ｐ１０５、ＩκＢα、ｃ－Ｍｙｃ、ＩＲＦ－１、ＨＩＶ－１、ＨＩＶ－２、ＳＩＶｍａｃ、ＣＭＶ、ＨＳＶ－１、ＳＶ４０、アデノウイルス等のプロモーターやそれらのコンセンサス配列を１個あるいは複数個有した合成プロモーター等が挙げられる。

【００７０】レポーター遺伝子を用いた検出方法では、上記プロモーターにレポーター遺伝子を連結した転写ユニットを作製した後、その転写ユニットを宿主細胞の染色体に組み込んだ細胞株を作製する。この細胞内に本発明のＤＮＡを発現するユニットを導入し本発明のＤＮＡを発現させた後、レポーター遺伝子の発現量を測定することにより、ＮＦ－κＢの活性化を検出できる。あるいは、上記プロモーターにレポーター遺伝子を連結した転写ユニットを作製した後、該転写ユニットと本発明のＤＮＡを発現するユニットの二つのユニットを同時に宿主細胞に導入し、レポーター遺伝子の発現量を測定することにより、ＮＦ－κＢの活性化を検出できる。

【００７１】３．本発明のポリペプチドの製造

本発明のポリペプチドは、モレキュラー・クローニング第２版やカレント・プロトコールズ・イン・モレキュラー・バイオロジー等に記載された方法等を用い、例えば以下の方法により、本発明のＤＮＡを宿主細胞中で発現させて、製造することができる。

【００７２】全長ｃＤＮＡをもとにして、必要に応じて、該ポリペプチドをコードする部分を含む適当な長さのＤＮＡ断片を調製する。該ＤＮＡ断片、または全長ｃＤＮＡを適当な発現ベクターのプロモーターの下流に挿入することにより、組換えベクターを作製する。該組換えベクターを、該発現ベクターに適合した宿主細胞に導入することにより、本発明のポリペプチドを生産する形

質転換体を得ることができる。

【００７３】宿主細胞としては、細菌、酵母、動物細胞、昆虫細胞、植物細胞等、目的とする遺伝子を発現できるものであればいずれも用いることができる。発現ベクターとしては、上記宿主細胞において自律複製可能ないしは染色体中への組込みが可能で、本発明のポリペプチドをコードするＤＮＡを転写できる位置にプロモーターを含有しているものが用いられる。

【００７４】細菌等の原核生物を宿主細胞として用いる場合は、本発明のポリペプチドをコードするＤＮＡを含有してなる組換えベクターは原核生物中で自律複製可能であると同時に、プロモーター、リボソーム結合配列、本発明のポリペプチドをコードする遺伝子、および転写終結配列より構成されたベクターであることが好ましい。尚、ベクターには、プロモーターを制御する遺伝子が含まれていてもよい。

【００７５】発現ベクターとしては、例えば、ｐＢＴｒｐ２（Ｂｏｅｈｒｉｎｇｅｒ Ｍａｎｎｈｅｉｍ社製）、ｐＢＴａｃ１（Ｂｏｅｈｒｉｎｇｅｒ Ｍａｎｎｈｅｉｍ社製）、ｐＢＴａｃ２（Ｂｏｅｈｒｉｎｇｅｒ Ｍａｎｎｈｅｉｍ社製）、ｐＫＫ233-2（Ｐｈａｒｍａｃｉａ社製）、ｐＳＥ280（Ｉｎｖｉｔｒｏｇｅｎ社製）、ｐＧＥＭＥＸ-1（Ｐｒｏｍｅｇａ社製）、ｐＱＥ-8（ＱＩＡＧＥＮ社製）、ｐＫＹＰ10（特開昭58-110600号）、ｐＫＹＰ200［Ａｇｒｉｃ ｕｌｔｕｒａｌ. Ｂｉｏｌｏｇｉｃａｌ. Ｃｈｅｍｉｓｔｒｙ., 48, 669 (1984)］、ｐＬＳＡ１［Ａｇｒｉｃ. Ｂｉｌ ｏ. Ｃｈｅｍ., 53, 277 (1989)］、ｐＧＥＬ１［Ｐｒｏｃ. Ｎａｔｌ. Ａｃａｄ. Ｓｃｉ. ＵＳＡ, 82, 4306 (1985)］、ｐＢｌｕｅｓｃｒｉｐｔ ＩＩ ＳＫ(-)（Ｓｔｒａｔａｇｅｎｅ社製）、ｐＴｒＳ30［Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＪＭ109/ｐＴｒＳ30（ＦＥＲＭ ＢＰ-5407）より調製］、ｐＴｒＳ32［Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＪＭ109/ｐＴｒＳ32（ＦＥＲＭ ＢＰ-5408）より調製］、ｐＣＨＡ２［Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＩＣＨＡ２（ＦＥＲＭ ＢＰ-400）より調製、特開昭60-221091号］、ｐＣＫＡ２［Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＩＣＫＡ２（ＦＥＲＭ ＢＰ-6798）より調製、特開昭60-221091号］、ｐＴｅｒｍ２（米国特許第4,686,191号、米国特許第4,939,094号、および米国特許第5,160,735号）、ｐＳｕｐｅｘ、ｐＵＢ110、ｐＴＰ５、ｐＣＩ９４、ｐＥＧ400［Ｊ. Ｂａｃｔｅｒｉｏｌ., 172, 2392 (1990)］、ｐＣＥＸ（Ｐｈａｒｍａｃｉａ社製）、ｐＥＴシステム（Ｎｏｖａｇｅｎ社製）等を挙げることができる.発現ベクターとしては、リボソーム結合配列であるシャインーダルガーノ(Ｓｈｉｎｅ-Ｄａｌｇａｒｎｏ)配列と開始コドンとの間を適当な距離（例えば6～18塩基）に調節したものを用いることが好ましい。

【００７６】プロモーターとしては、宿主細胞中で発現できるものであればいかなるものでもよい。例えば、ｔｒｐプロモーター（Ｐｔｒｐ）、ｌａｃプロモーター、Ｐｌプロモーター、Ｐｒプロモーター、Ｔ７プロモーター等の、大腸菌やファージ等に由来するプロモーターおよび、ＳＰＯ１プロモーター、ＳＰＯ２プロモーター、ｐｅｎＰプロモーター等を挙げることができる。また、Ｐｔｒｐを２つ直列させたプロモーター（Ｐｔｒｐ×２）、ｔａｃプロモーター、ｌａｃＴ７プロモーター、ｌｅｔＩプロモーター［Ｇｅｎｅ, 44, 29 (1986)］のように人為的に設計改変されたプロモーター等も用いること

ができる。

【００７７】本発明のポリペプチドをコードする部分の塩基配列を、宿主の発現に最適なコドンとなるように、塩基を置換することにより、目的とするポリペプチドの生産率を向上させることができる。本発明の組換えベクターにおいては、本発明のＤＮＡの発現には転写終結配列は必ずしも必要ではないが、構造遺伝子の直下に転写終結配列を配置することが好ましい。

【００７８】宿主細胞としては、エシェリヒア属、セラチア属、バチルス属、ブレビバクテリウム属、コリネバクテリウム属、ミクロバクテリウム属、シュードモナス属等に属する微生物、例えば、Ｅｓｃｈｅｒｉ ｃｈｉａ ｃｏｌｉ ＸＬ1-Ｂｌｕｅ、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＸＬ2-Ｂｌｕｅ、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＤＨ1、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＭＣ1000、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＫＹ3276、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ Ｗ1485、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ＪＭ109、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉＨＢ101、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ Ｎｏ. 49、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ Ｗ3110、Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉＮＹ49、Ｓｅｒｒ ａｔｉａ ｆｉｃａｒｉａ、Ｓｅｒｒａｔｉａ ｆｏｎｔｉｃｏｌａ、Ｓｅｒｒａｔｉａ ｌｉｑｕｅｆａｃｉｅｎｓ、Ｓｅｒｒａｔｉａ ｍａｒｃｅｓｃｅｎｓ、Ｂａｃｉ ｌｌｕｓ ｓｕｂｔｉｌｌｉｓ、Ｂａｃｉｌｌｕｓ ａｍｙｌｏｌｉｑｕｅｆａｃｉｎｅｓ、Ｂｒｅｖｉｂａｃｔｅｒｉｕｍ ａｍｍｏｎｉａｇｅｎｅｓ、Ｂｒｅｖｉｂａｃｔｅｒｉｕｍ ｉｍｍａｒｉｏｐｈｉｌｕｍ ＡＴＣＣ14068、Ｂｒｅｖｉｂａｃｔｅｒｉｕｍ ｓａｃｃｈａｒｏｌｙｔｉｃｕｍ ＡＴＣＣ14066、Ｂｒｅｖｉｂａｃｔｅｒｉｕｍ ｆｌａｖｕｍ ＡＴＣＣ14067、Ｂｒｅｖｉｂａｃｔｅｒｉｕｍ ｌａｃｔｏｆｅｒｍｅｎｔｕｍ ＡＴＣＣ13869、Ｃｏｒｙｎｅｂａｃｔｅｒｉｕｍ ｇｌｕｔａｍｉｃｕｍ ＡＴＣＣ13032、Ｍｉｃｒｏｂａｃｔｅｒｉｕｍ ａｍｍｏｎｉａｐｈｉｌｕｍ ＡＴＣＣ15354、Ｐｓｅｕｄｏｍｏｎａｓｕ ｓｐ. Ｄ-0110等を挙げることができる。

【００７９】組換えベクターの導入方法としては、上記宿主細胞へＤＮＡを導入する方法であればいずれも用いることができ、例えば、カルシウムイオンを用いる方法［Ｐｒｏｃ. Ｎａｔｌ. Ａｃａｄ. Ｓｃｉ. ＵＳＡ, 69, 2110 (1972)］、プロトプラスト法（特開昭63-248394号）、または、Ｇｅｎｅ, 17, 107 (1982) やＭｏｌｅｃｕｌａｒ & Ｇｅｎｅｒａｌ Ｇｅｎｅｔｉｃｓ, 168, 111 (1979) に記載の方法等を挙げることができる。

【００８０】酵母を宿主細胞として用いる場合には、発現ベクターとして、例えば、ＹＥＰ13（ＡＴＣＣ37115）、ＹＥｐ24（ＡＴＣＣ37051）、ＹＣｐ50（ＡＴＣＣ37419）、ｐＨＳ19、ｐＨＳ15等を挙げることができる。プロモーターとしては、酵母菌株中で発現できるものであればいずれのものを用いてもよく、例えば、ヘキソースキナーゼ等の解糖系の遺伝子のプロモーター、ＰＨＯ５プロモーター、ＰＧＫプロモーター、ＧＡＰプロモーター、ＡＤＨプロモーター、ｇａｌ１プロモーター、ｇａｌ１０プロモーター、ヒートショックタンパク質プロモーター、ＭＦ１ プロモーター、ＣＵＰ１プロモーター等を挙げることができる。

【００８１】宿主細胞としては、サッカロミセス属、クリュイベロミセス属、トリコスポロン属、シュワニオミセス属等に属する微生物、例えば、Ｓａｃｃａｒｏｍｙｃｅｓ ｃｅｒｅｖｉｓｉａｅ、Ｓｃｈｉｚｏｓａｃｃｈａｒｏｍｙｃｅｓ ｐｏｍｂｅ、Ｋｌｕｙｖｅｒｏｍｙｃｅ ｓ ｌａｃｔｉｓ、Ｔｒｉｃｈｏｓｐｏｒｏｎ ｐｕｌｌｕｌａｎｓ、Ｓｃｈｗａｎｎｉｏｍｙｃｅｓ ａｌｌ

uvius等を挙げることができる。組換えベクターの導入方法としては、酵母にＤＮＡを導入する方法であればいずれも用いることができ、例えば、エレクトロポレーション法（Methods. Enzymol., 194, 182 (1990)）、スフェロプラスト法（Proc. Natl. Acad. Sci. USA,84, 1929 (1978)）、酢酸リチウム法（J. Bacteriology, 153, 163 (1983)）、（Proc. Natl. Acad. Sci. USA, 75, 1929 (1978)）記載の方法等を挙げることができる。

【００８２】動物細胞を宿主として用いる場合には、発現ベクターとして、例えば、pcDNAI,pcDN8（フナコシ社製）、pAGE107〔特開平3-22979；Cytotechnology, 3, 133(1990)〕、pAS3-3（特開平2-227075）、pCDN8（Nature, 329, 840 (1987)）、pcDNAI/Amp（Invitrogen社製）、pREP4（Invitrogen社製）、pAGE103（J. Biochemistry, 101, 1307 (1987)）、pAGE210等を挙げることができる。

【００８３】プロモーターとしては、動物細胞中で発現できるものであればいずれも用いることができ、例えば、サイトメガロウイルス（ＣＭＶ）のＩＥ（immediate early）遺伝子のプロモーター、ＳＶ４０の初期プロモーター、レトロウイルスのプロモーター、メタロチオネインプロモーター、ヒートショックプロモーター、ＳＲαプロモーター等を挙げることができる。また、ヒトＣＭＶのＩＥ遺伝子のエンハンサーをプロモーターと共に用いてもよい。

【００８４】宿主細胞としては、ヒトの細胞であるナマルバ（Ｎａｍａｌｗａ）細胞、サルの細胞であるＣＯＳ細胞、チャイニーズ・ハムスターの細胞であるＣＨＯ細胞、ＨＢＴ５６３７（特開昭63-299）等を挙げることができる。組換えベクターの導入方法としては、動物細胞にＤＮＡを導入する方法であればいずれも用いることができ、例えば、エレクトロポレーション法〔Cytotechnology, 3, 133 (1990)〕、リン酸カルシウム法（特開平2-227075）、リポフェクション法（Proc. Natl. Acad. Sci. USA, 84, 7413 (1987)）等を挙げることができる。

【００８５】昆虫細胞を宿主として用いる場合には、例えばカレント・プロトコールズ・イン・モレキュラー・バイオロジー・サプリメント1-38 (1 987-1997)、Baculovirus Expression Vectors, A Laboratory Manual, W. H.Freeman and Company,NewYork (1992)、Bio/Technology, 6, 47 (1988)等に記載された方法によって、本発明のポリペプチドを発現することができる。

【００８６】即ち、組換え遺伝子導入ベクターおよびバキュロウイルスを昆虫細胞に共導入して昆虫細胞培養上清中に組換えウイルスを得た後、さらに組換えウイルスを昆虫細胞に感染させ、本発明のポリペプチドを発現させることができる。該方法において用いられる遺伝子導入ベクターとしては、例えば、pVL1392、pVL1393、pBlueBacIII（ともにInvitorogen社製）等を挙げることができる。

【００８７】バキュロウイルスとしては、例えば、夜盗蛾科昆虫に感染するウイルスであるアウトグラファ・カリフォルニカ・ヌクレアー・ポリヘドロシス・ウイルス（Autographa californica nuclear polyhedrosis virus)等を用いることができる。昆虫細胞としては、Spodoptera frugiperdaの卵巣細胞であるＳｆ９、Ｓｆ２１〔Baculovirus Expression Vectors, A Laboratory Manual, W. H. Freeman and Company, New York (1992)〕、Trichoplusia niの卵巣細胞であるＨｉｇｈ５（Invitrogen社製）等を用いることができる。

【００８８】組換えウイルスを調製するための、昆虫細胞への上記組換え遺伝子導入ベクターと上記バキュロウイルスの共導入方法としては、例えば、リン酸カルシウム法（特開平2-2270 75）、リポフェクション法（Proc. Natl. Acad. Sci. USA, 84,7413 (1987)）等を挙げることができる。植物細胞を宿主細胞として用いる場合には、発現ベクターとして、例えば、Ｔｉプラスミド、タバコモザイクウイルスベクター等を挙げることができる。

【００８９】プロモーターとしては、植物細胞中で発現できるものであればいずれのものを用いてもよく、例えば、カリフラワーモザイクウイルス（ＣａＭＶ）の３５Ｓプロモーター、イネアクチン１プロモーター等を挙げることができる。宿主細胞としては、タバコ、ジャガイモ、トマト、ニンジン、ダイズ、アブラナ、アルファルファ、イネ、コムギ、オオムギ等の植物細胞等を挙げることができる。

【００９０】組換えベクターの導入方法としては、植物細胞にＤＮＡを導入する方法であればいずれも用いることができ、例えば、アグロバクテリウム（Agrobacterium）（特開昭59-140885、特開昭60-70080、WO94/00977）、エレクトロポレーション法（特開昭60-251887）、パーティクルガン（遺伝子銃）を用いる方法（特許第2606856、特許第2517813）等を挙げることができる。

【００９１】遺伝子の発現方法としては、直接発現以外に、モレキュラー・クローニング第２版に記載されている方法等に準じて、分泌生産、融合ポリペプチド発現等を行うことができる。酵母、動物細胞、昆虫細胞または植物細胞により発現させた場合には、糖あるいは糖鎖が付加されたポリペプチドを得ることができる。

【００９２】本発明のＤＮＡを組み込んだ組換え発現ベクターを保有する形質転換体を培地に培養し、培養物中に本発明のポリペプチドを生成蓄積させ、該培養物より該ポリペプチドを採取することにより、該ポリペプチドを製造することができる。大腸菌等の原核生物あるいは酵母等の真核生物を宿主として得られた形質転換体を培養する培地としては、該生物が資化し得る炭素源、窒素源、無機塩類等を含有し、形質転換体の培養を効率的に行える培地であれば天然培地、合成培地のいずれを用いてもよい。

【００９３】炭素源としては、該生物が資化し得るものであればよく、グルコース、フラクトース、スクロース、これらを含有する糖蜜、デンプンあるいはデンプン加水分解物等の炭水化物、酢酸、プロピオン酸等の有機酸、エタノール、プロパノール等のアルコール類等を用いることができる。窒素源としては、アンモニア、塩化アンモニウム、硫酸アンモニウム、酢酸アンモニウム、リン酸アンモニウム等の無機酸もしくは有機酸のアンモニウム塩、その他の含窒素化合物、ならびに、ペプトン、肉エキス、酵母エキス、コーンスチープリカー、カゼイン加水分解物、大豆粕および大豆粕加水分解物、各種発酵菌体およびその消化物等を用いることができる。

【００９４】無機塩としては、リン酸第一カリウム、リン酸第二カリウム、リン酸マグネシウム、硫酸マグネシウム、塩化ナトリウム、硫酸第一鉄、硫酸マンガン、硫酸銅、炭酸カルシウム等を用いることができる。培養は、通常振盪培養または深部通気撹拌培養等の好気的条件下で行う。培養温度は１５～４０℃がよく、培養時間は、通常１６時間～７日間である。培養中のｐＨは３．０～９．０に保持する。ｐＨの調整は、無機または有機の酸、アルカリ溶液、尿素、炭酸カルシウム、アンモニア等を用いて行う。

【００９５】また、培養中必要に応じて、アンピシリンやテトラサイクリン等の抗生物質を培地に添加してもよい。プロモーターとして誘導性のプロモーターを用いた組換えベクターで形質転換した微生物を培養するときには、必要に応じてインデューサーを培地に添加してもよい。例えば、lacプロモーターを用いた組換えベクターで形質転換した微生物を培養するときにはイソプロピル－β－Ｄ－チオガラクトピラノシド（IPTG）等を、trpプロモーターを用いた組換えベクターで形質転換した微生物を培養するときにはインドールアクリル酸（ＩＡＡ）等を培地に添加してもよい。

【００９６】動物細胞を宿主として得られた形質転換体を培養する培地としては、一般に使用されているＲＰＭＩ１６４０培地〔The Journal of the American Medical Association, 199, 519 (1967)〕、ＥａｇｌｅのＭＥＭ培地〔Science, 122, 501(1952)〕、ダルベッコ改変ＭＥＭ培地〔Virology, 8, 396 (1959)〕、１９９培地〔Proceeding of the Society for the Biological Medicine, 73, 1 (1950)〕またはこれら培地に牛胎児血清等を添加した培地等を用いることができる。培養は、通常ｐＨ６～８、３０～４０℃、５％CO_2存在下等の条件下で１～７日間行う。また、培養中必要に応じて、カナマイシン、ペニシリン等の抗生物質を培地に添加してもよい。

【００９７】昆虫細胞を宿主として得られた形質転換体を培養する培地としては、一般に使用されているTNM-FH培地（Pharmingen社製）、Ｓｆ－９００ ＩＩ ＳＦＭ培地（Life Technologies社製）、ＥｘＣｅｌｌ４００、

ＥｘＣｅｌｌ４０５（いずれもJRH Biosciences社製）、Ｇｒａｃｅ'ｓ Ｉｎｓｅｃｔ Ｍｅｄｉｕｍ（Nature, 195, 788 (1962)）等を用いることができる。培養は、通常ｐＨ６～７、２５～３０℃等の条件下で、１～５日間行う。また、培養中必要に応じて、ゲンタマイシン等の抗生物質を培地に添加してもよい。

【００９８】植物細胞を宿主として得られた形質転換体は、細胞として、または植物の細胞や器官に分化させて培養することができる。該形質転換体を培養する培地としては、一般に使用されているムラシゲ・アンド・スクーグ（ＭＳ）培地、ホワイト（Ｗｈｉｔｅ）培地、またはこれら培地にオーキシン、サイトカイニン等、植物ホルモンを添加した培地等を用いることができる。培養は、通常ｐＨ５～９、２０～４０℃の条件下で３～６０日間行う。また、培養中必要に応じて、カナマイシン、ハイグロマイシン等の抗生物質を培地に添加してもよい。

【００９９】本発明のポリペプチドの生産方法としては、宿主細胞内に生産させる方法、宿主細胞外に分泌させる方法、あるいは宿主細胞外膜上に生産させる方法があり、使用する宿主細胞や、生産させるポリペプチドの構造を変えることにより、該方法を選択することができる。本発明のポリペプチドが宿主細胞内あるいは宿主細胞外膜上に生産される場合、ポールソンらの方法（J. Biol. Chem., 264, 17619 (1989)）、ロウらの方法（Proc. Natl. Acad. Sci. USA, 86, 8227 (1989)、Genes Develop., 4, 1288 (1990)）、または特開平5-336963、WO94/23021等に記載の方法を準用することにより、該ポリペプチドを宿主細胞外に積極的に分泌させることができる。

【０１００】すなわち、遺伝子組換えの手法を用いて、本発明のポリペプチドの活性部位を含むポリペプチドの手前にシグナルペプチドを付加した形で発現させることにより、本発明のポリペプチドを宿主細胞外に積極的に分泌させることができる。また、特開平2-227075に記載されている方法に準じて、ジヒドロ葉酸還元酵素遺伝子等を用いた遺伝子増幅系を利用して生産量を上昇させることもできる。

【０１０１】さらに、遺伝子導入した動物または植物の細胞を再分化させることにより、遺伝子が導入された動物個体（トランスジェニック非ヒト動物）または植物個体（トランスジェニック植物）を造成し、これらの個体を用いて本発明のポリペプチドを製造することもできる。形質転換体が動物個体または植物個体の場合は、通常の方法に従って、飼育または栽培し、該ポリペプチドを生成蓄積させ、該動物個体または植物個体より該ポリペプチドを採取することにより、該ポリペプチドを製造することができる。

【０１０２】動物個体を用いて本発明のポリペプチドを製造する方法としては、例えば公知の方法〔American Journal of Clinical Nutrition, 63, 639S (1996)、Ame

rican Journal of Clinical Nutrition, 63, 627S (1996)、Bio/Technology, 9, 830 (1991)〕に準じて遺伝子を導入して造成した動物中に本発明のポリペプチドを生産する方法が挙げられる。

【0103】動物個体の場合は、例えば、本発明のポリペプチドをコードするDNAを導入したトランスジェニック非ヒト動物を飼育し、該ポリペプチドを該動物中に生成・蓄積させ、該動物中より該ポリペプチドを採取することにより、該ポリペプチドを製造することができる。該動物中の蓄積場所としては、例えば、該動物のミルク（特開昭63-309192）、卵等を挙げることができる。この際に用いられるプロモーターとしては、動物で発現できるものであればいずれも用いることができるが、例えば、乳腺細胞特異的なプロモーターであるαカゼインプロモーター、βカゼインプロモーター、βラクトグロブリンプロモーター、ホエー酸性プロテインプロモーター等が好適に用いられる。

【0104】植物個体を用いて本発明のポリペプチドを製造する方法としては、例えば本発明のポリペプチドをコードするDNAを導入したトランスジェニック植物を公知の方法〔組織培養, 20 (1994)、組織培養, 21 (1995)、Trends in Biotechnology, 15, 45 (1997)〕に準じて栽培し、該ポリペプチドを該植物中に生成・蓄積させ、該植物中より該ポリペプチドを採取することにより、該ポリペプチドを生産する方法が挙げられる。

【0105】本発明の形質転換体により製造されたポリペプチドは、例えば本発明のポリペプチドが、細胞内に溶解状態で発現した場合には、培養終了後、細胞を遠心分離により回収し、水系緩衝液にけん濁後、超音波破砕機、フレンチプレス、マントンガウリンホモゲナイザー、ダイノミル等により細胞を破砕し、無細胞抽出液を得る。該無細胞抽出液を遠心分離することにより得られる上清から、通常の酵素の単離精製法、即ち、溶媒抽出法、硫安等による塩析法、脱塩法、有機溶媒による沈殿法、ジエチルアミノエチル（DEAE）ーセファロース、DIAIONHPA-75（三菱化成社製）等レジンを用いた陰イオン交換クロマトグラフィー法、S-Sepharose FF (Pharmacia社製) 等のレジンを用いた陽イオン交換クロマトグラフィー法、ブチルセファロース、フェニルセファロース等のレジンを用いた疎水性クロマトグラフィー法、分子篩を用いたゲルろ過法、アフィニティークロマトグラフィー法、クロマトフォーカシング法、等電点電気泳動等の電気泳動法等の手法を単独あるいは組み合わせて用い、精製標品を得ることができる。

【0106】また、該ポリペプチドが細胞内に不溶体を形成して発現した場合は、同様に細胞を回収後破砕し、遠心分離を行うことにより、沈殿画分としてポリペプチドの不溶体を回収する。回収したポリペプチドの不溶体をタンパク質変性剤で可溶化する。該可溶化液を希釈または透析することにより、該ポリペプチドを正常な立体構造に戻した後、上記と同様の単離精製法により該ポリペプチドの精製標品を得ることができる。

【0107】本発明のポリペプチドあるいはその糖修飾体等の誘導体が細胞外に分泌された場合には、培養上清に該ポリペプチドあるいはその糖鎖付加体等の誘導体を回収することができる。即ち、該培養物を上記と同様の遠心分離等の手法により処理することにより可溶性画分を取得し、該可溶性画分から、上記と同様の単離精製法を用いることにより、精製標品を得ることができる。

【0108】また、本発明のポリペプチドは、Fmoc法（フルオレニルメチルオキシカルボニル法）、tBoc法（t-ブチルオキシカルボニル法）等の化学合成法によっても製造することができる。また、Advanced ChemTech社、Perkin-Elmer社、Amersham Pharmacia Biotech社、Protein Technology Instrument社、Synthecell-Vega社、PerSeptive社、島津製作所等のペプチド合成機を利用して化学合成することもできる。

【0109】4．本発明のポリペプチドを認識する抗体の調製

本発明のポリペプチドまたは該ポリペプチドの部分断片ポリペプチドの精製標品、あるいは本発明のポリペプチドの一部のアミノ酸配列を有する合成ペプチドを抗原として用いることにより、ポリクローナル抗体、モノクローナル抗体等、本発明のポリペプチドを認識する抗体を作製することができる。

【0110】（1）ポリクローナル抗体の作製

本発明のポリペプチドの全長または該ポリペプチドの部分断片ポリペプチドの精製標品、あるいは本発明のポリペプチドの一部のアミノ酸配列を有するペプチドを抗原として用い、適当なアジュバント〔例えば、フロイントの完全アジュバント(Complete Freund's Adjuvant)または水酸化アルミニウムゲル、百日咳ワクチン等〕とともに、動物の皮下、静脈内または腹腔内に投与することによりポリクローナル抗体を作製することができる。

【0111】投与する動物として、ウサギ、ヤギ、3～20週令のラット、マウス、ハムスター等を用いることができる。該抗原の投与量は動物1匹当たり50～100μgが好ましい。ペプチドを用いる場合は、ペプチドをスカシガイヘモシアニン（keyhole limpet haemocyanin）や牛チログロブリン等のキャリア蛋白に共有結合させたものを抗原とするのが望ましい。抗原とするペプチドは、ペプチド合成機で合成することができる。

【0112】該抗原の投与は、1回目の投与の後、1～2週間おきに3～10回行う。各投与後、3～7日目に眼底静脈叢より採血し、該血清が免疫に用いた抗原と反応することを酵素免疫測定法（酵素免疫測定法（ELISA法）：医学書院刊（1976年）、Antibodies-A Laboratory Manual, Cold Spring Harbor Laboratory (1988)〕等で確認する。

【0113】免疫に用いた抗原に対し、その血清が充分な抗体価を示した非ヒト哺乳動物より血清を取得し、該血清を分離、精製することによりポリクローナル抗体を取得することができる。分離、精製する方法としては、遠心分離、40～50%飽和硫酸アンモニウムによる塩析、カプリル酸沈殿〔Antibodies, A Laboratory manual, Cold SpringHarbor Laboratory, (1988)〕、またはDEAEーセファロースカラム、陰イオン交換カラム、プロテインAまたはGーカラムあるいはゲル濾過カラム等を用いるクロマトグラフィー等を、単独または組み合わせて処理する方法が挙げられる。

【0114】（2）モノクローナル抗体の作製

(a) 抗体産性細胞の調製

免疫に用いた本発明のポリペプチドの部分断片ポリペプチドに対し、その血清が十分な抗体価を示したラットを抗体産生細胞の供給源として供する。該抗体価を示したラットに抗原物質を最終投与した後3～7日目に、脾臓を摘出する。

【0115】該脾臓をMEM培地（日水製薬社製）中で細断し、ピンセットでほぐし、1，200rpmで5分間遠心分離した後、上清を捨てる。得られた沈殿画分の脾細胞をトリスー塩化アンモニウム緩衝液（pH7．65）で1～2分間処理し赤血球を除去した後、MEM培地で3回洗浄し、得られた脾細胞を抗体産生細胞として用いる。

【0116】(b) 骨髄腫細胞の調製

骨髄腫細胞としては、マウスまたはラットから取得した株化細胞を使用する。例えば、8ーアザグアニン耐性マウス（BALB／c由来）骨髄腫細胞株P3-X63Ag8-U1（以下、P3-U1と略す）〔Curr. Topics. Microbiol. Immunol., 81, 1 (1978)、Europ. J. Immunol., 6, 511 (1976)〕、SP2／0-Ag14（SP-2）〔Nature, 276, 269 (1978)〕、P3-X63-Ag8653（653）〔J. Immunol., 123, 1548 (1979)〕、P3-X63-Ag8（X63）〔Nature, 256, 495 (1975)〕等を用いることができる。これらの細胞株は、8ーアザグアニン培地〔RPMI-1640培地にグルタミン（1．5mmol／l）、2ーメルカプトエタノール（5×10^{-5}mol／l）、ジェンタマイシン（10μg／ml）および牛胎児血清（FCS）（CSL社製、10%）を加えた培地（以下、正常培地という）に、さらに8ーアザグアニン（15μg／ml）を加えた培地〕で継代するが、細胞融合の3～4日前に正常培地で培養し、融合には該細胞を2×10^{7}個以上用いる。

【0117】(c) ハイブリドーマの作製

(b)で取得した抗体産生細胞と(b)で取得した骨髄腫細胞をMEM培地またはPBS（リン酸二ナトリウム1．83g、リン酸一カリウム0．21g、食塩7．65g、蒸留水1リットル、pH7．2）でよく洗浄し、細胞数が、抗体産生細胞：骨髄腫細胞＝5～10：1になるよう混合し、1，200rpmで5分間遠心分離した後、上清を捨てる。

【0118】得られた沈殿画分の細胞群をよくほぐし、該細胞群に、撹拌しながら、37℃で、10^{8}抗体産生細胞あたり、ポリエチレングリコール-1000（PEG-1000）2g、MEM 2mlおよびジメチルスルホキシド（DMSO）0．7mlを混合した溶液を0．2～1ml添加し、さらに1～2分間毎にMEM培地1～2mlを数回添加する。

【0119】添加後、MEM培地を加えて全量が50mlになるように調製する。該調製液を900rpmで5分間遠心分離後、上清を捨てる。得られた沈殿画分の細胞を、ゆるやかにほぐした後、メスピペットによる吸込み、吹出しでゆるやかにHAT培地〔正常培地にヒポキサンチン（10^{-4}mol／l）、チミジン（1．5×10^{-5}mol／l）およびアミノプテリン（4×10^{-7}mol／l）を加えた培地〕100ml中に懸濁する。

【0120】該懸濁液を96穴培養用プレートに100μl／穴ずつ分注し、5%CO_2インキュベーター中、37℃で7～14日間培養する。培養後、培養上清の一部をとりアンチボディイズ〔Antibodies, A Laboratory manual, Cold Spring Harbor Laboratory , Chapter 14 (1988)〕等に述べられている酵素免疫測定法により、本発明のポリペプチドの部分断片ポリペプチドに特異的に反応するハイブリドーマを選択する。

【0121】酵素免疫測定法の具体的例として、以下の方法を挙げることができる。免疫の際、抗原に用いた本発明のポリペプチドの部分断片ポリペプチドを適当なプレートにコートし、ハイブリドーマ培養上清もしくは後述の（d）で得られる精製抗体を第一抗体として反応させ、さらに第二抗体としてビオチン、酵素、化学発光物質あるいは放射線化合物等で標識した抗ラットまたは抗マウスイムノグロブリン抗体を反応させた後に標識物質に応じた反応を行ない、本発明のポリペプチドに特異的に反応するものを本発明のモノクローナル抗体を生産するハイブリドーマとして選択する。

【0122】該ハイブリドーマを用いて、限界希釈法によりクローニングを2回繰り返し〔1回目は、HT培地（HAT培地からアミノプテリンを除いた培地）、2回目は、正常培地を使用する〕、安定して強い抗体価の認められたものを本発明のモノクローナル抗体を産生するハイブリドーマ株として選択する。

(d) モノクローナル抗体の調製

プリスタン処理〔2，6，10，14ーテトラメチルペンタデカン（Pristane）0．5mlを腹腔内投与し、2週間飼育する〕した8～10週令のマウスまたはヌードマウスに、（c）で取得した本発明のポリペプチドに対するモノクローナル抗体産生ハイブリドーマ細胞5～20×10^{6}細胞／匹を腹腔内に注射する。10～21日間でハイブリドーマは腹水癌化する。

【０１２３】胰腹水癌化したマウスから腹水を採取し、３，０００ｒｐｍで５分間遠心分離して固形分を除去する。得られた上清より、ポリクローナルで用いた方法と同様の方法でモノクローナル抗体を精製、取得することができる。抗体のサブクラスの決定は、マウスモノクローナル抗体タイピングキットまたはラットモノクローナル抗体タイピングキットを用いて行う。タンパク質量は、ローリー法あるいは２８０ｎｍでの吸光度より算出する。

【０１２４】５.本発明のポリペプチドを生産する組換えウイルスベクターの調製法

以下に、本発明のポリペプチドを特定のヒト組織内で生産するための組換えウイルスベクターの調製法について述べる。本発明のＤＮＡの完全長ｃＤＮＡをもとに、必要に応じて、該ポリペプチドをコードする部分を含む適当な長さのＤＮＡ断片を調製する。

【０１２５】完全長ｃＤＮＡ、あるいは該ＤＮＡ断片をウイルスベクター内のプロモーターの下流に挿入することにより、組換えウイルスベクターを造成する。ＲＮＡウイルスベクターの場合には、本発明のＤＮＡの完全長ｃＤＮＡに相同なｃＲＮＡ，若しくは該ポリペプチドをコードする部分を含む適当な長さのＤＮＡ断片に相同なＲＮＡ断片を調整し、それらを、ウイルスベクター内のプロモーターの下流に挿入することにより、組換えウイルスを造成する。ＲＮＡ断片は、２本鎖の他、ウイルスベクターの種類に応じて、センス鎖若しくはアンチセンス鎖のどちらか一方の１本鎖を選択する。例えば、レトロウイルスベクターの場合は、センス鎖に相同するＲＮＡを、センダイウイルスベクターの場合は、逆にアンチセンス鎖に相同なＲＮＡを選択する。

【０１２６】該組換えウイルスベクターを、該ベクターに適合したパッケージング細胞に導入する。パッケージング細胞はウイルスのパッケジーングに必要なポリペプチドをコードするＤＮＡの少なくとも１つを欠損している組換えウイルスベクターの該欠損するポリペプチドを補給できる細胞は全て用いることができ、例えばヒト腎臓由来のＨＥＫ２９３細胞、マウス繊維芽細胞ＮＩＨ３Ｔ３等を用いることができる。パッケージング細胞で補給するポリペプチドとしては、レトロウイルスベクターの場合はマウスレトロウイルス由来のｇａｇ、ｐｏｌ、ｅｎｖ等のポリペプチドが、レンチウイルスベクターの場合はＨＩＶウイルス由来のｇａｇ、ｐｏｌ、ｅｎｖ、ｖｐｒ、ｖｐｕ、ｖｉｆ、ｔａｔ、ｒｅｖ、ｎｅｆ等のポリペプチド、アデノウイルスベクターの場合はアデノウイルス由来のＥＩＡ、ＥＩＢ等のポリペプチドが、アデノ随伴ウイルスの場合はＲｅｐ（ｐ５、ｐ１９、ｐ４０）、Ｖｐ（Ｃａｐ）等のポリペプチドが、センダイウイルスの場合はＮＰ、Ｐ／Ｃ、Ｌ、Ｍ、Ｆ、ＨＮ等のポリペプチドが挙げられる。

【０１２７】ウイルスベクターとしては上記パッケージング細胞において組換えウイルスが生産でき、標的細胞で本発明のＤＮＡを転写できる位置にプロモーターを含有しているものが用いられる。プラスミドベクターとしてはWFG（Proc. Natl. Acad.Sci. USA, 92, 6733-6737 (19 95)）、pBabePuro（Nucleic Acids Res., 18, 3587 -3596 (1990)）、LL-CG、CL-CG、CS-CG、CLG（Journal of Virology, 72, 8150-8157 (1998)）、pAdexl（Nucleic Acids Res., 23, 3816-3821 (1995)）等が用いられる。

【０１２８】プロモーターとしては、ヒト組織中で発現できるものであればいずれも用いることができ、例えば、サイトメガロウイルス（ヒトＣＭＶ）のＩＥ（immediateearly）遺伝子のプロモーター、ＳＶ４０の初期プロモーター、レトロウイルスのプロモーター、メタロチオネインプロモーター、ヒートショックタンパク質プロモーター、ＳＲαプロモーター等を挙げることができる。また、ヒトＣＭＶのＩＥ遺伝子のエンハンサーをプロモーターと共に用いてもよい。

【０１２９】パッケージング細胞への組換えウイルスベクターの導入法としては、例えば、リン酸カルシウム法（特開平2-227075号公報）、リポフェクション法（Proc. Natl. Acad. Sci. U SA, 84, 7413 (1987)）等を挙げることができる。

６．本発明のＤＮＡ、ポリペプチドまたは抗体の利用

（１）本発明のＤＮＡの発現を検出する方法

本発明のＤＮＡを用いて、検体における本発明のＤＮＡのｍＲＮＡ発現量、該ｍＲＮＡの構造変化を検出することができる。

【０１３０】検体としては、本発明のＤＮＡの発現変化が原因となっている疾患を有する患者ならびに健常者より取得した組織、血清、唾液等の生体試料、該生体試料から細胞を取得して試験管内の適当な培地中で培養した初代培養細胞試料、または生体試料から取得した組織を、パラフィンあるいはクリオスタット切片として単離したもの等から取得したｍＲＮＡあるいは全ＲＮＡ等が用いられる（以後、該ｍＲＮＡおよび全ＲＮＡを検体由来ＲＮＡと称する）。

【０１３１】検出する方法としては、例えば（１）ノーザンブロット法（２）in situハイブリダイゼイション法、（３）定量的ＰＣＲ法、（４）デファレンシャル・ハイブリダイゼイション法（Trends in Genetics 7, 31 4, (1991)）、（５）ＤＮＡチップ法（Genome Researc h, 6, 639, (1996)）、（６）ＲＮａｓｅ保護アッセイ法等の方法等が挙げられる。以下、各検出法について詳述する。

【０１３２】①ノーザンブロット法

検体由来ＲＮＡをゲル電気泳動で分離後、ナイロンフィルター等の支持体に転写する。転写後、本発明のＤＮＡより調製した標識プローブを用いて、ハイブリダイゼイションならびに洗浄を行う。洗浄後、該プローブと特異的に結合したＲＮＡのバンドを検出する。健常者と患者由来の検体ＲＮＡについて該検出結果を比較することにより、該ＲＮＡの発現量ならびに構造の変化を検出することができる。ハイブリダイゼイションを行う際には、プローブと検体由来ＲＮＡ中の目的とするｍＲＮＡが安定なハイブリッドを形成する条件でインキュベーションする。偽陽性を防ぐためには、ハイブリダイゼイションならびに洗浄工程をモレキュラー・クローニング第２版に記載の方法に準じて高ストリンジェントな条件で行うことが望ましい。

【０１３３】ノーザンブロット法に用いる標識プローブは、例えば、公知の方法(ニック・トランスレーション、ランダム・プライミングまたはキナージング)により放射性同位体、ビオチン、蛍光基、化学発光基等を、本発明のＤＮＡあるいは該ＤＮＡの配列から設計したオリゴヌクレオチドに取り込ませることで調製できる。標識プローブのｍＲＮＡへの結合量は該ｍＲＮＡの発現量を反映することから、結合した標識プローブの量を定量することで該ｍＲＮＡの発現量を定量することができる。また、標識プローブが結合するフィルター上の部位を分析することで、該ｍＲＮＡの構造変化を知ることができる。

【０１３４】②in situハイブリダイゼイション法

生体から取得した組織をパラフィンあるいはクリオスタット切片として単離して得られた検体、および①記載の標識プローブを用いてハイブリダイゼイションならびに洗浄の工程を行う。洗浄後、①と同様の方法により該プローブと特異的に結合したｍＲＮＡの発現量を検出することができる。in situハイブリダイゼイション法で、偽陽性を防ぐためには、ハイブリダイゼイションならびに洗浄工程をカレント・プロトコールズ・イン・モレキュラー・バイオロジー等に記載されている方法に準じて高ストリンジェントな条件で行うことが望ましい。

【０１３５】③定量的ＰＣＲ法

検体由来ＲＮＡ、オリゴｄＴプライマーまたはランダムプライマー、および逆転写酵素を用い、ｃＤＮＡを合成することに基づいた方法を用いることにより目的とするＲＮＡを検出することができる（以後、該ｃＤＮＡを検体由来ｃＤＮＡと称する）。検体由来ＲＮＡがｍＲＮＡの場合は、上記①のいずれのプライマーも用いることができるが、該検体由来ＲＮＡが全ＲＮＡである場合は、オリゴｄＴプライマーを用いることが必要である。

【０１３６】定量的ＰＣＲ法では、検体由来ｃＤＮＡをテンプレートとし本発明のＤＮＡが有する塩基配列に基づき設計したプライマーを用いてＰＣＲを行うことで、特定のｍＲＮＡ由来のＤＮＡ断片が増幅される。該増幅ＤＮＡ断片の量は該ｍＲＮＡの発現量を反映することから、アクチンやＧ３ＰＤＨ(glyceraldehyde 3-phosphat e dehydrogenase)等をコードするＤＮＡを内部コントロールとして置くことで該ｍＲＮＡの量を定量することが可能である。また、該増幅ＤＮＡ断片をゲル電気泳動により分離することで、該ｍＲＮＡの構造の変化を知ることもできる。本検出法では、標的配列を特異的にかつ効率的に増幅する適当なプライマーを用いることが望ましい。適当なプライマーは、プライマー間の結合やプライマー内の結合を起こさず、アニーリング温度で標的ｃＤＮＡと特異的に結合して、変性条件で標的ｃＤＮＡからはずれる等の条件に基づき設計することができる。増幅ＤＮＡ断片の定量は増幅産物が指数関数的に増加しているＰＣＲ反応の内に行うことが必要である。このようなＰＣＲ反応は、各反応ごとに生産される該増幅ＤＮＡ断片を回収してゲル電気泳動で定量分析することで知ることができる。

【０１３７】④デファレンシャル・ハイブリダイゼイション法およびＤＮＡチップ法

③に記載された方法で調製した検体由来ｃＤＮＡをプローブとして、本発明のＤＮＡを固定化させたフィルターあるいはスライドガラスやシリコン等の基盤に対してハイブリダイゼイションならびに洗浄を行う。洗浄後、本発明のＤＮＡと特異的に結合したｃＤＮＡ量を測定することにより該ｃＤＮＡ由来のｍＲＮＡの発現量の変動を検出することができる。デファレンシャル・ハイブリダイゼイション法およびＤＮＡチップ法のいずれの方法もフィルターあるいは基盤上にアクチンやＧ３ＰＤＨ等の内部コントロールを固定化することで、対照検体と標的検体の間での該ｍＲＮＡの発現の違いを正確に検出することができる。また対照検体と標的検体由来のＲＮＡをもとにそれぞれ異なる標識ｄＮＴＰを用いて標識ｃＤＮＡ合成を行い、１枚のフィルターあるいは１枚の基盤に二つの標識ｃＤＮＡプローブを同時にハイブリダイズさせることで正確な該ｍＲＮＡの発現量の定量を行うことができる。

【０１３８】⑤ＲＮａｓｅ保護アッセイ法

本発明のＤＮＡの３'端にＴ７プロモーター、ＳＰ６プロモーター等のプロモーター配列を結合し、ＲＮＡポリメラーゼを用いたin vitroの転写系により標識したｒＮＴＰを用いて、標識したアンチセンスＲＮＡを合成する。該標識アンチセンスＲＮＡを、検体由来ＲＮＡと結合させて、ＲＮＡ－ＲＮＡハイブリッドを形成させた後、ＲＮａｓｅで消化し、消化から保護されたＲＮＡ断片をゲル電気泳動によりバンドを形成させ検出する。得られたバンドを定量することで、上記標識アンチセンスＲＮＡと結合するｍＲＮＡの発現量を定量することができる。

【０１３９】尚、①～⑤のいずれかに記載した方法に用いられるＤＮＡとしては、例えば配列番号６～１０のいずれかで表される塩基配列を有するＤＮＡもしくはそれらから得られるＤＮＡ断片等が挙げられる。また、当該方法による検出に供する検体としては、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、

エンドトキシンショック、敗血症、微生物感染、慢性B型肝炎、慢性C型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、外傷性脳損傷、炎症性腸疾患等の感染や炎症を伴う疾患、バーキットリンパ腫、ホジキン病、各種リンパ腫、成人T細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、関節リウマチ、変形性関節炎等の異常な線維芽細胞や滑膜組織の活性化を伴う疾患、エイズ等のウイルス性疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄等の平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群（ＳＩＲＳ：systemic inflammatory response syndrome）、成人呼吸窮迫症候群（ＡＲＤＳ：adultrespiratory distress syndrome）等の疾患が挙げられ、当該検出方法により本発明のＤＮＡの発現を検出することで、上記疾患の診断に利用することができる。

【０１４０】（２）本発明のＤＮＡの変異を検出する方法

以下、被験者における本発明のＤＮＡの変異の有無を検出する方法について述べる。被験者における該ＤＮＡの変異は本発明のＤＮＡと下記方法により直接比較することにより検出することができる。被験者から、組織、血液、唾液等のヒト生体試料あるいは、該生体試料から樹立した初代培養細胞由来の試料を集め、該生体試料あるいは該初代培養細胞由来試料中からＤＮＡを抽出する（以下、該ＤＮＡを検体由来ＤＮＡと称する）。または、該試料由来のｍＲＮＡより常法によりｃＤＮＡを取得する（以下、該ｃＤＮＡを検体由来ｃＤＮＡと称する）。該検体由来ＤＮＡまたはｃＤＮＡを鋳型とし、本発明のＤＮＡが有する塩基配列に基づき設計したプライマーを用いてＰＣＲ法等によりＤＮＡを増幅する。得られた増幅ＤＮＡを試料ＤＮＡとして用いる。

【０１４１】増幅ＤＮＡに変異があるかどうかを検出する方法として、野生型対立遺伝子を有するＤＮＡ鎖と変異対立遺伝子を有するＤＮＡ鎖とのハイブリダイズにより形成されるヘテロ二本鎖を検出する方法を用いることができる。ヘテロ二本鎖を検出する方法には、①ポリアクリルアミドゲル電気泳動によるヘテロ二本鎖検出法（Trends Genet., 7, 5 (1991)）、②一本鎖コンフォメーション多型解析法（Genomics, 16, 325-332 (1993)）、③ミスマッチの化学的切断法（CCM, chemical cleavage of mismatches）（Human Molecular Genetics (1996), Tom Strachan and Andre w P. Read (BIOS Scientific Publishers Limited)）、④ミスマッチの酵素的切断法（Nature Genetics, 9, 103-104 (1996)）、⑤変性ゲル電気泳動法（Mutat. Res., 288, 103-112 (1993)）⑥タンパク質短縮試験（protein truncation test：ＰＴＴ法）（Genomics, 20, 1-4 (1994)）等の方法が挙げられる。以下、上記方法について説明する。

【０１４２】①ポリアクリルアミドゲル電気泳動によるヘテロ二本鎖検出法

検体由来ＤＮＡあるいは検体由来ｃＤＮＡをテンプレートに、該ＤＮＡを配列番号６～１０のいずれかに記載の塩基配列に基づき設計したプライマーにより、２００ｂｐよりも小さいＤＮＡ断片として増幅する。本発明のＤＮＡおよび被験者由来の該増幅ＤＮＡ断片を用い、各々の増幅ＤＮＡ断片による２本鎖形成処理を常法により行う。処理後、ポリアクリルアミドゲル電気泳動を行う。該ＤＮＡの変異によりヘテロ二本鎖が形成された場合は、変異を持たないホモ二本鎖よりも移動度が遅く、それらはホモ二本鎖とは別のバンドとして検出することができる。特製のゲル（Hydro-link,MDEなど）を用いた方が分離度はよい。２００ｂｐよりも小さい断片の検索ならば、挿入、欠失、ほとんどの１塩基置換を検出可能である。ヘテロ二本鎖解析は、次に述べる一本鎖コンフォメーション多型解析と組み合わせた１枚のゲルで行うことが望ましい。

【０１４３】②一本鎖コンフォメーション多型解析法

一本鎖コンフォメーション多型解析（ＳＳＣＰ解析；single strand conformation polymorphism analysis）では、検体由来ＤＮＡあるいは検体由来ｃＤＮＡをテンプレートに、配列番号６～１０のいずれかに記載の塩基配列に基づき設計したプライマーにより、２００ｂｐよりも小さい断片として増幅した該ＤＮＡを変性後、未変性ポリアクリルアミドゲル中で電気泳動する。ＤＮＡ増幅を行う際にプライマーを放射性同位体あるいは蛍光色素で標識し、該標識を指標とするか、または未標識の増幅産物を電気泳動後、銀染色することにより、増幅した該ＤＮＡをバンドとして検出することができる。本発明のDNA由来の増幅DNA断片と、被験者由来のものとを同時に電気泳動することにより、変異を持った断片を移動度の違いから検出できる。

【０１４４】③ミスマッチの化学的切断法

ミスマッチの化学的切断法（ＣＣＭ法）では、検体由来ＤＮＡあるいは検体由来ｃＤＮＡをテンプレートに、該ＤＮＡを配列番号６～１０のいずれかに記載の塩基配列に基づき設計したプライマーで増幅したＤＮＡ断片を、本発明のＤＮＡに放射性同位体あるいは蛍光色素をとり込ませた標識ＤＮＡとハイブリダイズさせ、四酸化オスミウムで処理することでミスマッチしている場所のＤＮＡの一方の鎖を切断させ変異を検出することができる。ＣＣＭ法は最も感度の高い検出法の１つであり、キロベースの長さの検体にも適応できる。

【０１４５】④ミスマッチの酵素的切断法

上記四酸化オスミウムの代わりにＴ４ファージリゾルベースとエンドヌクレアーゼＶＩＩのような細胞内でミスマッチの修復に関与する酵素とＲＮａｓｅＡと組み合わせることで、酵素的にミスマッチを切断することもできる。

⑤変性ゲル電気泳動法

変性ゲル電気泳動法（denaturing gradient gel electrophoresis：ＤＧＧＥ法）では、検体由来ＤＮＡあるいは検体由来ｃＤＮＡをテンプレートに、配列番号６～１０のいずれかに記載の塩基配列に基づき設計したプライマーで増幅したＤＮＡ断片を化学的変性剤の濃度勾配や温度勾配を有するゲルを用いて電気泳動する。増幅したＤＮＡ断片はゲル内を一本鎖に変性する位置まで移動し、変性後は移動しなくなる。該ＤＮＡに変異がある場合とない場合では増幅したＤＮＡのゲル内での移動度が異なることから、変異の存在を検出することが可能である。検出感度を上げるにはそれぞれのプライマーにポリ（Ｇ：Ｃ）端末を付けるとよい。

【０１４６】⑥ タンパク質短縮試験（protein truncation test：ＰＴＴ法）

該試験によりポリペプチドの欠損を生み出すフレームシフト突然変異、スプライス部位突然変異、ナンセンス突然変異を特異的に検出することができる。ＰＴＴ法では、配列番号６～１０のいずれかに表された塩基配列を有するＤＮＡの５’末端にＴ７プロモーター配列と真核生物翻訳開始配列をつないだ特殊なプライマーを設計し、該プライマーを用いて検体由来ＲＮＡより逆転写ＰＣＲ（ＲＴ－ＰＣＲ）法でｃＤＮＡを作成する。該ｃＤＮＡを用い、in vitro転写、翻訳を行うと、ポリペプチドが生産される。該ポリペプチドをゲルに泳動して、該ポリペプチドの泳動位置が完全長ポリペプチドに相当する位置にあれば欠損を生み出す変異は存在せず、該ポリペプチドに欠損がある場合は、完全長ポリペプチドより短い位置に該ポリペプチドは泳動され、該位置より欠損の程度を知ることができる。

【０１４７】上記の方法で変異が検出された場合には、本発明のＤＮＡが有する塩基配列に基づいて設計したプライマーを用い、常法により変異を有する検体由来ＤＮＡならびに検体由来ｃＤＮＡの塩基配列を決定することが可能である。決定された塩基配列を解析することにより、検体由来ＤＮＡあるいは検体由来ｃＤＮＡが特定の疾患を有する被験者の場合には、該疾患の原因となる変異を特定できる。以後、該変異を検出することにより、疾患の診断に利用することが出来る。

【０１４８】上記方法により検出されるＤＮＡのコード領域における変異以外の変異の検出には、該ＤＮＡの付近、該ＤＮＡ中のイントロンおよび調節配列のような非コード領域を検査することによって検出し得る。非コード領域中の変異に起因する疾患は、上記に記載した方法に従い対照検体と比較した場合の、疾患患者における異常なサイズの、または異常な生産量のｍＲＮＡを検出することで確認することができる。

【０１４９】このようにして非コード領域における変異の存在が示唆された該ＤＮＡについては、配列番号６～１０のいずれかに記載の塩基配列を有するＤＮＡをハイブリダイゼイションのプローブとして用いることにより、クローン化することができる。非コード領域における変異は上述のいずれかの方法に準じて探索することができる。

【０１５０】見い出された変異は、Handbook of Human Genetics Linkage. The John Hop kins University Press, Baltimore (1994) に記載された方法に従い統計処理を行うことで、疾患との連鎖があるＳＮＰｓ（シングル・ヌクレオチド・ポリモルフィズム）として同定することができる。上記変異を検出する方法で診断可能な被験者としては、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エンドトキシンショック、敗血症、微生物感染、慢性B型肝炎、慢性C型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、外傷性脳損傷、炎症性腸疾患等の感染や炎症を伴う疾患、バーキットリンパ腫、ホジキン病、各種リンパ腫、成人Ｔ細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、関節リウマチ、変形性関節炎等の異常な線維芽細胞や滑膜組織の活性化を伴う疾患、エイズ等のウイルス性疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄などの平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群（ＳＩＲＳ：systemic inflammatory response syndrome）、成人呼吸窮迫症候群（ＡＲＤＳ：adult respiratory distress syndrome）等のいずれかの疾患を有する者を挙げることができる。

【０１５１】（３）本発明のＤＮＡまたはオリゴヌクレオチドを用いて本発明のポリペプチドをコードするＤＮＡの転写または翻訳を抑制する方法

アンチセンスＲＮＡ／ＤＮＡ技術〔バイオサイエンスとインダストリー，50,322 (1992)、化学，46，681 (1991)、Biotechnology，9，358 (1992)、Trends in Biotechnology，10，87 (1992)、Trends in Biotechnology，10，152 (1992)、細胞工学，16，1463 (1997)〕、トリプル・ヘリックス技術〔Trends in Biotechnology，10，132 (1992)〕等により、本発明のＤＮＡを利用して、本発明のポリペプチドをコードするＤＮＡの転写または翻訳を抑制することができる。例えば、本発明のＤＮＡまたはオリゴヌクレオチドを、本発明のポリペプチドを発現できる系（生体を含む）に共存させ、該ポリペプチドの発現を転写、翻訳レベルで抑制できる。

【０１５２】該抑制方法は、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エンドトキシンショック、敗血症、微生物感染、慢性B型肝炎、慢性C型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、

外傷性脳損傷、炎症性腸疾患等の感染や炎症を伴う疾患、バーキットリンパ腫、ホジキン病、各種リンパ腫、成人T細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、関節リウマチ、変形性関節炎等の異常な線維芽細胞や滑膜組織の活性化を伴う疾患、エイズ等のウイルス性疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄等の平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群（ＳＩＲＳ：systemic in flammatory response syndrome）、成人呼吸窮迫症候群（ＡＲＤＳ：adult respiratory distress syndrome）等、本発明のポリペプチドをコードするＤＮＡの変異が原因となっている疾患の治療または予防に利用することができる。

【０１５３】（４）本発明のＤＮＡまたはオリゴヌクレオチドを用いて本発明のポリペプチドをコードするＤＮＡのプロモーター領域および転写制御領域を取得する方法

本発明のＤＮＡまたはオリゴヌクレオチドをプローブとして用い、公知の方法〔モレキュラー・クローニング第２版、東京大学医科学研究所制癌研究部編,新細胞工学実験プロトコール,秀潤社（1993年）〕により、本発明のポリペプチドをコードするＤＮＡのプロモーター領域および転写制御領域を取得することが可能である。例えば、以下の方法で、ラットあるいはヒト由来のものを取得することができる。

【０１５４】ラットあるいはヒトの細胞や組織から単離した染色体ＤＮＡを用いて作製したゲノムＤＮＡライブラリーに対して、本発明のＤＮＡまたはオリゴヌクレオチド（特にｃＤＮＡの5'側の部分）をプローブとして、プラークハイブリダイゼーション等の方法でスクリーニングする。該スクリーニングにより、ハイブリダイズするゲノムＤＮＡを取得する。該ＤＮＡよりプロモーター領域および転写制御領域を得ることができる。また、得られたゲノムＤＮＡの塩基配列とｃＤＮＡの塩基配列を比較することによりエキソン／イントロン構造を明らかにすることができる。

【０１５５】尚、同様の方法を用いて、他の非ヒト哺乳動物においても該ＤＮＡのプロモーター領域および転写制御領域を取得することができる。プロモーター領域としては、哺乳動物細胞において本発明のポリペプチドをコードするＤＮＡの基本転写に関与する領域が挙げられ、転写制御領域としては、本発明のポリペプチドをコードするＤＮＡの基本転写を増強するエンハンサー配列および減弱するサイレンサー配列等を含む領域が挙げられる。例えば、ヒトの骨髄で、本発明のポリペプチドをコードするＤＮＡの転写に関与するプロモーター領域および転写制御領域を挙げることができる。得られたプロモーターおよび転写制御領域は後述のスクリーニング方法に利用することができる他、該ＤＮＡの転写の制御機構を解析するために有用である。

【０１５６】（５）本発明のポリペプチドをコードするＤＮＡを用いたスクリーニングにより、該ＤＮＡの転写を制御する医薬を取得する方法

患者由来の細胞株に種々の被験化合物を添加し、本発明のＤＮＡを用いて、ｍＲＮＡの発現の増減を検定することで該ＤＮＡの転写もしくは翻訳を抑制または促進する物質をスクリーニングすることができる。該ＤＮＡのｍＲＮＡの発現の増減は、上記したＰＣＲ法、ノーザンブロット法、ＲＮａｓｅ保護アッセイ法により検出できる。

【０１５７】患者由来細胞株に種々の被験化合物を添加し、本発明のポリペプチドを特異的に認識する抗体を用いて、該ポリペプチドの発現の増減を検定することで該ＤＮＡの転写もしくは翻訳を促進する物質をスクリーニングすることができる。該ポリペプチドの発現の増減は、上記した蛍光抗体法、酵素免疫測定法（ＥＬＩＳＡ法）、放射性物質標識免疫抗体法（ＲＩＡ）、免疫組織染色法、免疫細胞染色法等の免疫組織化学染色法（ＡＢＣ法、ＣＳＡ法等）、ウェスタンブロッティング法、ドットブロッティング法、免疫沈降法、サンドイッチＥＬＩＳＡ法により検出できる。

【０１５８】また、本発明のポリペプチドをコードするＤＮＡのプロモータ領域および転写制御領域の下流に、クロラムフェニコールアセチルトランスフェラーゼ（ＣＡＴ）遺伝子やルシフェラーゼ遺伝子をレポーター遺伝子として連結したレポータープラスミドを構築し、適当な細胞宿主に導入して形質転換体を得た後、その形質転換体に種々の被験物質を添加し、レポーター遺伝子の発現の増減を解析することにより、本発明のポリペプチドをコードするＤＮＡの発現を転写レベルで制御する医薬をスクリーニングすることができる。

【０１５９】（６）本発明のポリペプチドを用いたスクリーニング方法により本発明のポリペプチドに作用する医薬を取得する方法。

本発明のポリペプチドあるいは該ポリペプチドの部分ペプチドを発現した形質転換体と種々の被験物質とを共存させ、該形質転換体におけるＮＦ－κＢの活性化の変動を解析することにより、本発明のポリペプチドに作用する医薬をスクリーニングすることができる。また、精製した該ポリペプチドあるいは該ポリペプチドの部分ペプチドも該ポリペプチドに特異的に作用する医薬のスクリーニングに利用することができる。該スクリーニングによって得られた物質は、本発明のＤＮＡおよびポリペプチドが関与した疾患の治療のための医薬として有用である。

【０１６０】以下、２種のスクリーニング法について説明する。

スクリーニング法（１）

本発明のポリペプチドあるいは該ポリペプチドの部分ペプチドを生産するように形質転換した微生物、動物細胞、または昆虫細胞（以後探索用形質転換体と称する）と被験物質とを水性媒体中で共存させる。共存後、上記２．に記載の方法に準じてＮＦ－κＢの活性を測定する。形質転換していない宿主の微生物、動物細胞、または昆虫細胞を対照群として比較し、該形質転換体におけるＮＦ－κＢの活性化の程度を変動させる被験物質を選択することで目的の物質を取得することができる。また、該探索用形質転換体に特異的に結合する化合物あるいはポリペプチドの、該探索用形質転換体に対する結合を阻害することを指標にして、上記と同様の方法により、標的化合物を競合スクリーニングすることができる。

【０１６１】精製した本発明のポリペプチドまたは該ポリペプチドの一部を構成するポリペプチドは、該ポリペプチドに特異的に結合する標的化合物を選択するのに用いることができる。標的化合物を定量するには、本発明のポリペプチドを特異的に認識する抗体を用いて上記の免疫学的方法により行うことができる。また、該ポリペプチドあるいは該ポリペプチドのポリペプチドに結合する標的化合物の結合を阻害することを指標に、標的化合物を競合スクリーニングすることができる。

【０１６２】スクリーニング法（２）

該ポリペプチドの一部を構成するペプチドを多数、プラスチックピンまたはある種の固体支持体上で高密度に合成し、該ペプチドに選択的に結合する化合物あるいはポリペプチドを効率的にスクリーニングすることができる（WO84/03564）。尚、本発明のポリペプチドを発現する形質転換体を用いて、遺伝子の発現を解析することにより、本発明のポリペプチドにより転写制御を受ける遺伝子をスクリーニングすることができる。

【０１６３】（７）本発明のＤＮＡ、または該ＤＮＡと相同な配列からなるＲＮＡを含有する遺伝子治療剤

本発明のＤＮＡ、または該ＤＮＡと相同な配列からなるＲＮＡを含有するウイルスベクターを用いた遺伝子治療剤は、上記の５．で作製した組換えウイルスベクターおよび遺伝子治療剤に用いる基剤を調合することにより製造することができる〔Nature Genet., 8, 42 (1994)〕。遺伝子治療剤に用いる基剤としては、通常注射剤に用いる基剤であればどのようなものでもよく、蒸留水、塩化ナトリウムまたは塩化ナトリウムと無機塩との混合物等の塩溶液、マンニトール、ラクトース、デキストラン、グルコース等の糖溶液、グリシン、アルギニン等のアミノ酸溶液、有機酸溶液又は塩溶液とグルコース溶液との混合溶液等があげられる。また常法に従い、これらの基剤に浸透圧調整剤、ｐＨ調整剤、ゴマ油、ダイズ油等の植物油又はレシチンもしくは非イオン界面活性剤等の界面活性剤等の助剤を用いて、溶液、懸濁液、分散液として注射剤を調製してもよい。これらの注射剤を、粉末化、凍結乾燥等の操作により用時溶解用製剤として調製することもできる。本発明の遺伝子治療剤は、液体の場合はそのままで、個体の場合は必要により滅菌処理をした上記の基剤に遺伝子治療の直前に溶解して治療に使用することができる。本発明の遺伝子治療剤の投与方法としては、患者の治療部位に吸収されるように、局所的に投与する方法をあげることができる。

【０１６４】適当なサイズの本発明のＤＮＡを、アデノウイルス・ヘキソン・タンパク質に特異的なポリリジン-コンジュゲート抗体と組み合わせてコンプレックスを作製し、得られたコンプレックスをアデノウイルスベクターに結合させることにより、ウイルスベクターを調製することができる。該ウイルスベクターは安定に標的細胞に到達し、エンドソームにより細胞内に取り込まれ、細胞内で分解され効率的にＤＮＡを発現させることができる。

【０１６５】（一）鎖ＲＮＡウイルスであるセンダイウイルスをベースにしたウイルスベクターも開発されており（特願平9-517213、特願平9-517214）、遺伝子治療を目的としてＫＲＧＦ－１遺伝子を組み込んだセンダイウイルスベクターを作製することができる。該ＤＮＡは、非ウイルス遺伝子移入法によっても病巣に輸送することができる。

【０１６６】当該分野で公知の非ウイルス遺伝子移入法には、リン酸カルシウム共沈法〔Virology, 52, 456-467 (1973)；Science, 209, 1414-1422 (1980)〕、マイクロインジェクション法〔Proc. Natl. Acad. Sci. USA, 77, 5399-5403 1980)；Proc. Natl. Acad. Sci. USA, 77, 7380-7384 (1980)；Cell, 27, 223-231 (1981)；Nature, 294, 92-94 (1981)〕、リポソームを介した膜融合-介在移入法〔Proc. Natl. Acad. Sci. USA, 84, 7413-7417 (1987)；Biochemistry, 28, 9508-9514 (1989)；J. Biol. Chem., 264, 12126-12129 (1989)；Hum. Gene Ther., 3,267-275 (1992)；Science, 249, 1285-1288 (1990)；Circulation, 83, 2007-2011 (1992)〕あるいは直接ＤＮＡ取り込みおよび受容体-媒介ＤＮＡ移入法〔Science, 247, 1465-1468 (1990)；J. Biol. Chem., 266, 14338-14342 (1991)；Proc. Natl. Acad. Sci. USA, 87, 3655-3659 (1991)；J. Biol. Chem., 264,16985-16987 (1989)；BioTechniques, 11, 474-485 (1991)；Proc. Natl. Acad.Sci. USA, 87, 3410-3414 (1990)；Proc. Natl. Acad. Sci. USA, 88, 4255-4259 (1991)；Proc. Natl. Acad. Sci. USA, 87, 4033-4037 (1990)；Proc. Natl. Acad. Sci. USA, 88, 8850-8854 (1991)；Hum . Gene Ther., 3, 147-154(1991)〕等を挙げることができる。

【０１６７】リポソームを介した膜融合ー介在移入法ではリポソーム調製物を標的とする組織に直接投与することにより、当該組織の局所的な遺伝子の取り込みおよび発現が可能であることが腫瘍に関する研究において報告されている〔Hum. Gene Ther., 3, 399-410 (1992)〕。

したがって同様の効果が本発明のＤＮＡおよびポリペプチドが関与する疾患病巣でも期待される。ＤＮＡを病巣に直接ターゲッティングするには、直接ＤＮＡ取り込み技術が好ましい。受容体-媒介ＤＮＡ移入は、例えば、ポリリジンを介して、ポリペプチドリガンドにＤＮＡ(通常、共有的に閉環したスーパーコイル化プラスミドの形態をとる)をコンジュゲートすることによって行う。リガンドは、標的細胞または組織の細胞表面上の対応するリガンド受容体の存在に基づいて選択する。当該リガンド-ＤＮＡコンジュゲートは、所望により、血管に直接注射することができ、受容体結合およびＤＮＡ-タンパク質コンプレックスの内在化が起こる標的組織に指向し得る。ＤＮＡの細胞内破壊を防止するために、アデノウイルスを同時感染させて、エンドソーム機能を崩壊させることもできる。

【０１６８】（８）本発明の抗体を用いて本発明のポリペプチドを免疫学的に検出する方法

本発明のポリペプチドを特異的に認識する抗体を用い、抗原抗体反応を行わせることにより、本発明のポリペプチドまたは該ポリペプチドを含む組織を免疫学的に検出することができる。該検出法は、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エンドトキシンショック、敗血症、微生物感染、慢性Ｂ型肝炎、慢性Ｃ型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、外傷性脳損傷、変形性関節炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、炎症性腸疾患等の感染や炎症を伴う疾患、バーキットリンパ腫、ホジキン病、各種リンパ腫、成人Ｔ細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、慢性関節リウマチ、肺線維症等の異常な線維芽細胞や滑膜組織の活性化を伴う疾患、エイズ等のウイルス性疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄等の平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群（ＳＩＲＳ：systemic inflammatory response syndrome）、成人呼吸窮迫症候群（ＡＲＤＳ：adult respiratory distress syndrome）等、本発明のポリペプチドをコードするＤＮＡの変異が原因となっている疾患の診断に利用することができる。また、該検出方法は、ポリペプチドの定量にも用いられる。

【０１６９】免疫学的に検出および定量する方法としては、蛍光抗体法、酵素免疫測定法（ＥＬＩＳＡ法）、放射性物質標識免疫抗体法（ＲＩＡ）、免疫組織染色法や免疫細胞染色法等の免疫組織化学染色法（ＡＢＣ法、ＣＳＡ法等）、ウェスタンブロッティング法、ドットブロッティング法、免疫沈降法、サンドイッチＥＬＩＳＡ法〔単クローン抗体実験マニュアル（講談社サイエンティフィック）(1987)、続生化学実験講座５,免疫生化学研究法（東京化学同人）(1986)〕等が挙げられる。

【０１７０】蛍光抗体法とは、本発明のポリペプチドを細胞内あるいは細胞外に発現した微生物、動物細胞あるいは昆虫細胞または組織に、本発明の抗体を反応させ、さらにフルオレシン・イソチオシアネート（ＦＩＴＣ）等の蛍光物質でラベルした抗マウスＩｇＧ抗体あるいはその断片を反応させた後、蛍光色素をフローサイトメーターで測定する方法である。

【０１７１】酵素免疫測定法（ＥＬＩＳＡ法）とは、該ポリペプチドを細胞内あるいは細胞外に発現した微生物、動物細胞あるいは昆虫細胞または組織に、本発明の抗体を反応させ、さらにペルオキシダーゼ、ビオチン等の酵素標識等を施した抗マウスＩｇＧ抗体あるいは結合断片を反応させた後、発色色素を吸光光度計で測定する方法である。

【０１７２】放射性物質標識免疫抗体法（ＲＩＡ）とは、該ポリペプチドを細胞内あるいは細胞外に発現した微生物、動物細胞あるいは昆虫細胞または組織に、本発明の抗体を反応させ、さらに放射線標識を施した抗マウスＩｇＧ抗体あるいはその断片を反応させた後、シンチレーションカウンター等で測定する方法である。免疫細胞染色法、免疫組織染色法とは、該ポリペプチドを細胞内あるいは細胞外に発現した微生物、動物細胞あるいは昆虫細胞または組織に、該ポリペプチドを特異的に認識する抗体を反応させ、さらにＦＩＴＣ等の蛍光物質、ペルオキシダーゼ、ビオチン等の酵素標識を施した抗マウスＩｇＧ抗体あるいはその断片を反応させた後、顕微鏡を用いて観察する方法である。

【０１７３】ウェスタンブロッティング法とは、該ポリペプチドを細胞内あるいは細胞外に発現した微生物、動物細胞あるいは昆虫細胞または組織の抽出液をＳＤＳ-ポリアクリルアミドゲル電気泳動〔Antibodies-A Laboratory Manual, Cold SpringHarbor Laboratory, (1988)〕で分画した後、該ゲルをＰＶＤＦ膜あるいはニトロセルロース膜にブロッティングし、該膜に本発明の該ポリペプチドを特異的に認識する抗体を反応させ、さらにＦＩＴＣ等の蛍光物質、ペルオキシダーゼ、ビオチン等の酵素標識を施した抗マウスＩｇＧ抗体あるいはその断片を反応させた後、確認する方法である。

【０１７４】ドットブロッティング法とは、該ポリペプチドを細胞内あるいは細胞外に発現した微生物、動物細胞あるいは昆虫細胞または組織の抽出液をニトロセルロース膜にブロッティングし、該膜に本発明の抗体を反応させ、さらにＦＩＴＣ等の蛍光物質、ペルオキシダーゼ、ビオチン等の酵素標識を施した抗マウスＩｇＧ抗体あるいは結合断片を反応させた後、確認する方法である。

【０１７５】免疫沈降法とは、本発明のポリペプチドを細胞内あるいは細胞外に発現した微生物、動物細胞あるいは昆虫細胞または組織の抽出液を該ポリペプチドを特異的に認識する抗体と反応させた後、プロテインＧ－セファロース等イムノグロブリンに特異的な結合能を有する担体を加えて抗原抗体複合体を沈降させる方法である。

【０１７６】サンドイッチＥＬＩＳＡ法とは、本発明のポリペプチドを特異的に認識する抗体で、抗原認識部位の異なる２種類の抗体のうち、あらかじめ一方の抗体をプレートに吸着させ、もう一方の抗体をＦＩＴＣ等の蛍光物質、ペルオキシダーゼ、ビオチン等の酵素で標識しておき、抗体吸着プレートに、該ポリペプチドを細胞内あるいは細胞外に発現した微生物、動物細胞あるいは昆虫細胞または組織の抽出液を反応させた後、標識した抗体を反応させ、標識物質に応じた反応を行う方法である。

【０１７７】（９）本発明のポリペプチドを特異的に認識する抗体を用いて疾患を診断する方法

ヒト生体試料ならびヒト初代培養細胞での、該ポリペプチドの発現量の変化ならびに発現しているポリペプチドの構造変化を同定することは、将来、疾患を発症する危険性や既に発症した疾患の原因を知る上で有用である。該ポリペプチドの発現量や構造変化を検出して診断する方法としては、上記した、蛍光抗体法、酵素免疫測定法（ＥＬＩＳＡ法）、放射性物質標識免疫抗体法（ＲＩＡ）、免疫組織染色法や免疫細胞染色法等の免疫組織化学染色法（ＡＢＣ法、ＣＳＡ法等）、ウェスタンブロッティング法、ドットブロッティング法、免疫沈降法、サンドイッチＥＬＩＳＡ法等が挙げられる。

【０１７８】上記方法による診断に供する検体としては、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エンドトキシンショック、敗血症、微生物感染、慢性Ｂ型肝炎、慢性Ｃ型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、外傷性脳損傷、変形性関節炎、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、炎症性腸疾患等の感染や炎症を伴う疾患、バーキットリンパ腫、ホジキン病、各種リンパ腫、成人Ｔ細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、慢性関節リウマチ、肺線維症等の異常な線維芽細胞や滑膜組織の活性化を伴う疾患、エイズ等のウイルス性疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄等の平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群（ＳＩＲＳ：systemic inflammatory response syndrome）、成人呼吸窮迫症候群（ＡＲＤＳ：adult respiratory distress syndrome）等、本発明のポリペプチドをコードするＤＮＡの変異が原因となっている疾患の患者より取得した組織、血液、血清、尿、便、唾液等の生体試料そのものあるいは、該生体試料から取得した細胞ならびに細胞抽出液が用いられる。また、生体試料から取得した組織を、パラフィンあるいはクリオスタット切片として単離したものを用いることもできる。

【０１７９】免疫学的に検出する方法としては、マイクロタイタープレートを用いるＥＬＩＳＡ法・蛍光抗体法、ウェスタンブロット法、免疫組織染色法等が挙げられる。免疫学的に定量する方法としては、液相中で本発明のポリペプチドと反応する抗体のうちエピトープが異なる２種類のモノクローナル抗体を用いたサンドイッチＥＬＩＳＡ法、^{125}Ｉ等の放射性同位体で標識した本発明のポリペプチドと本発明のポリペプチドを認識する抗体とを用いるラジオイムノアッセイ法等が挙げられる。

【０１８０】（１０）本発明のＤＮＡを用いたノックアウト非ヒト動物の作製

本発明のＤＮＡを含有してなる組換えベクターを用い、目的とする非ヒト動物、例えばウシ、ヒツジ、ヤギ、ブタ、ウマ、マウス、ニワトリ等の胚性幹細胞(embryonic stem cell)において、染色体上の本発明のポリペプチドをコードするＤＮＡを公知の相同組換えの手法〔例えば、Nature, 326, 295 (1987)、Cell, 51, 503 (1987)等〕により不活化または任意の配列と置換した変異クローンを作製する〔例えば、Nature, 350, 243 (1991)〕。胚性幹細胞の変異クローンを用い、動物の受精卵の胚盤胞(blastcyst)への注入キメラ法または集合キメラ法等の手法により、胚性幹細胞クローンと正常細胞からなるキメラ個体を調製することができる。このキメラ個体と正常個体の掛け合わせにより、全身の細胞の染色体上の本発明のポリペプチドをコードするＤＮＡに任意の変異を有する個体を得ることができ、さらにその個体の掛け合わせにより相同染色体の双方に変異が入ったホモ個体の中から、本発明のポリペプチドをコードするＤＮＡの発現が一部または完全に抑制された個体としてノックアウト非ヒト動物を得ることができる。

【０１８１】また、染色体上の本発明のポリペプチドをコードするＤＮＡの任意の位置へ変異を導入することにより、ノックアウト非ヒト動物を作製することも可能である。例えば染色体上の本発明のポリペプチドをコードするＤＮＡの翻訳領域中へ塩基を置換、欠失、挿入等させて変異を導入することにより、その産物の活性を改変させることも可能である。また、その発現制御領域への同様な変異を導入することにより、発現の程度、時期、組織特異性等を改変させることも可能である。さらにＣｒｅ－ｌｏｘＰ系との組合せにより、より積極的に発現時期、発現部位、発現量等を制御することも可能である。このような例として、脳のある特定の領域で発現されるプロモータを利用して、その領域でのみ目的遺伝子を欠失させた例（Cell, 87, 1317, (1996)）やＣｒｅを発現するアデノウィルスを用いて、目的の時期に、臓器特異的に目的遺伝子を欠失させた例（Science, 278, 5335(1997)）が知られている。

【０１８２】従って、染色体上の本発明のポリペプチド

をコードするDNAについても、このように任意の時期や組織で発現を制御できる、または任意の挿入、欠失、置換をその翻訳領域や発現制御領域に有する、ノックアウト非ヒト動物を作製することができる。ノックアウト非ヒト動物は、任意の時期、任意の程度または任意の部位で、本発明のポリペプチドに起因する種々の疾患の症状を誘導することができる。このように、本発明のノックアウト非ヒト動物は、本発明のポリペプチドに起因する種々の疾患の治療や予防において極めて有用な動物モデルとなる。特にその治療薬、予防薬、また機能性食品、健康食品等の評価用モデルとして非常に有用である。

【0183】7．本発明のポリペプチドの変異導入および機能改変変異体の選択

（1）本発明のポリペプチドの変異導入

該ポリペプチドに変異を導入する方法としては、欠失・挿入・置換のいかなる方法を用いてもよい。ポリペプチドの欠失・挿入は、該ポリペプチドをコードするDNAをモレキュラークローニング第2版やカレント・プロトコールズ・イン・モレキュラー・バイオロジー等に記載された方法により当なDNA断片を欠失させる、あるいは適当なDNA断片を挿入させることにより可能である。

【0184】例えば、欠失変異体であれば、該DNAの中で適当な同じあるいは異なる制限酵素サイトを2個見出し、該DNAを含んだプラスミド等を市販の該制限酵素により消化後、平滑末端であればそのまま、突出末端であればKlenow Fragment（TaKaRa社製）等のDNAポリメラーゼにより平滑化し、再連結させることにより得ることができる。挿入変異体であれば、平滑末端化後に適当な二本鎖DNAを挿入し、連結させることにより得ることができる。置換変異体は、ランダムに変異を導入する方法として、例えばError Prone PCR法〔Trends In Biotechnology, 16, 76 (1998)）〕等を用いることができる。目的の位置に変異を導入する方法として、変異を有したプライマーを用いたPCR法（Mutagenesis and Synthesis of Novel Recombinant Genes Using PCR, PCR PRIMER A LABORATORY MANUAL,603 (1994)）あるいはQuikChange™ Site-Directed Mutagenesis Kit (STRATAGENE社製）等を用いることができる。

【0185】（2）本発明のポリペプチドの機能改変変異体の選択

（1）で作製した該ポリペプチドの変異体より、上記2.に記載した方法に準じて、NF－κB活性化に対する活性上昇改変変異体の選択が可能である。具体的には、該ポリペプチドおよび該ポリペプチドの変異体のそれぞれをレポーター細胞に導入し、該ポリペプチドよりレポーター活性を上昇させた変異体を選択することにより、NF－κB活性化機能を上昇した機能改変変異体を得ることができる。また、NF－κBを活性化する刺激

存在下でNF－κB活性化を抑制する該ポリペプチドの変異体を選択することにより、ドミナントネガティブ変異体を得ることができる。

【0186】具体的には、該ポリペプチドの変異体をレポーター細胞に導入し、サイトカイン（TNF－α、TNF－β、IL－1α、IL－1β、IL－2、LIF等）、T細胞マイトジェン（抗原刺激、レクチン、抗T細胞レセプター抗体、抗CD2抗体、抗CD3抗体、抗CD28抗体、Caイオノフォア）、B細胞マイトジェン（抗IgM抗体、anti－CD40）、ロイコトリエン、LPS、PMA、寄生体感染、ウイルス感染（HIV－1、HTLV－1、HBV、EBV、CMV、HSV－1、HHV－6、NDV、センダイウイルス、アデノウイルス等）、ウイルス産物（二本鎖RNA、Tax、HBX、EBNA－2、LMP－1等）、DNA破壊物質類、タンパク質合成インヒビター類（例えばシクロヘキシミド）、紫外線、放射線、酸化ストレス等のNF－κBを活性化する刺激を与え、レポーター活性が変異体を導入していない時よりも低下した該ポリペプチドの変異体を選択することにより、ドミナントネガティブ変異体を得ることができる。

【0187】尚、得られたドミナントネガティブ変異体（Dominant Negative mutants；優性機能抑制変異体）は、炎症応答抑制や悪性細胞の増殖抑制に応用可能であり、該ドミナントネガティブ変異体をコードするDNAは、NF－κBの活性化を伴う疾患の遺伝子治療に利用できる可能性がある。以下に実施例をあげて、本発明を具体的に説明する。ただし、これらの実施例は説明のためのものであり、本発明の技術的範囲を制限するものではない。

【0188】

【実施例】〔実施例1〕ヒト大腸およびヒト脂肪組織由来完全長cDNAライブラリーの作製

ヒトの大腸および脂肪組織より、モレキュラー・クローニング第2版に記載の方法によりmRNAを抽出した。さらに、オリゴdTセルロースでpolyA+RNAを精製した。それぞれのpolyA+ RNAよりオリゴキャップ法〔Gene, 138, 171-174 (1994)〕によりcDNAライブラリーを作製した。Oligo-cap linker（配列番号11）およびOligo dT primer（配列番号12）を用い、蛋白質核酸酵素, 41, 197-201 (1996)またはGene, 200, 149-156 (1997)に記載の方法に従って、BAP（Bacterial Alkaline Phosphatase）処理、TAP（Tobacco Acid Phosphatase）処理、RNAライゲーション、第一鎖cDNAの合成とRNAの除去を行った。得られた第一鎖cDNAを鋳型として、5'末端側のセンスプライマー（配列番号13）と3'末端側のアンチセンスプライマー（配列番号14）の2種のプライマーを用いるPCRにより二本鎖cDNAを増幅し、SfiIで切断した。PCRは、市販のキット：GeneAmp XL PCRキット（Perkin Elmer社製）を使用して、95℃で5分間熱処理後、95℃で1分間、58℃で1分間および72℃で10分間の反応サイクルを12回繰り返し、その後4℃で保持することにより行った。

【0189】DraIIIで切断したベクターpME18SFL3（GeneBank AB009864、発現ベクター，3392bp）に上記増幅cDNAを挿入し、cDNAライブラリーを作製した。得られたクローンのプラスミドDNA各々について、cDNAの5'端と3'端の塩基配列を、DNAシークエンシング試薬（Dye Terminator Cycle SequencingFS Ready Reaction Kit, dRhodamine Terminator Cycle Sequencing FS ReadyReaction KitまたはBigDye Terminator Cycle Sequencing FS Ready ReactionKit, PE Biosystems社製）を用い、マニュアルにしたがってシークエンス反応を行った後、DNAシークエンサー（ABI PRISM 377, PE Biosystems社製）を用いて塩基配列を決定した。

【0190】〔実施例2〕NF－κBエンハンサーによりルシフェラーゼ活性が発現制御されるレポーター細胞株の樹立

IFN－β中のNF－κB認識配列（配列番号15）を3回繰り返した人工プロモーターを作製し、ルシフェラーゼレポーターベクター（pAGE-luc:特開平3-22979、実験医学，7, 96-103 （1989））のルシフェラーゼ遺伝子の5'上流域に挿入した（以後、pIF-lucとよぶ）。該プラスミド 4μgを1μg/μlとなるようにTE緩衝液〔10 mmol/lトリスーHCl（pH8.0）、1 mmol/l EDTA（エチレンジアミン4酢酸ナトリウム）〕に溶解し、エレクトロポレーション法（BIO-RAD社製：Gene Pulser™）によって、ヒト腎細胞株293（Clontech社製）1.6×10⁶個に遺伝子導入した。pIF-lucは、ハイグロマイシン（Hygromycin）耐性遺伝子を含んでおり、遺伝子導入後は、ハイグロマイシン0.2g/lを添加したRPMI培地〔RPMI1640（日本水産社製）、10% 子牛血清、0.05 mmol/l－メルカプトエタノール、25 U/ml ペニシリンG、25U/ml ストレプトマイシン〕で培養、ハイグロマイシンを遺伝子導入の選択マーカーとして安定形質転換株を樹立した。安定形質転換株のうち、TNF－α刺激によって、無刺激と比較して670倍という高いルシフェラーゼ活性を誘導した株を選択（以後、293/IF-LUCとよぶ）し、以下の発現アッセイに用いた。

【0191】〔実施例3〕293/IF－LUCを用いた完全長DNAのNF－κB活性化に対する解析

実施例1で塩基配列を決定したクローンを、アンピシリン（100 mg/l）を添加した2×YT培地（Yeast ex tract 10 g/l、Trypton 16 g/l、NaC 5g/l）2ml中、37℃で，16時間、各々振盪培養した。培養後、菌体を遠心分離機で回収し、プラスミド調製キット（QIAPrep96 Turbo Miniprep Kit, QIAGEN社製）を用いて添付資料の方法で各々プラスミドを調製した。96ウエルプレートに293/IF－LUC細胞を1ウエルあたり20,000個となるように分注し、37℃で，16時間、CO_2インキュベーター中で培養した。この培養細胞に、上記プラスミド約0．25μgをそれぞれリポフェクション試薬（LIPOFECT AMINE 2000™ Reagent, GIBCO BRL社製）を用いて、添付資料の方法に従って導入した。37℃で，16時間、CO_2インキュベーター中で培養後、ルシフェラーゼ活性測定試薬（LucLite™, Packar社製）とルシフェラーゼ活性測定装置（ARVO 1420 MULTILABEL COUNTER, WALLC社製）を用いて、ルシフェラーゼ活性を測定した。

【0192】その結果、COL03279（配列番号6の塩基配列を有するDNAクローン）、COL06772（配列番号7の塩基配列を有するDNAクローン）、ADKA01604（配列番号8の塩基配列を有するDNAクローン）、ADSU00701（配列番号9の塩基配列を有するDNAクローン）、CAS01989（配列番号10の塩基配列を有するDNAクローン）の各クローンのプラスミドを導入した場合において、それぞれネガティブコントロール（pME18SFL3を使用）と比較して12．5倍、6．3倍、4．4倍、2．7倍、3．0倍の活性が確認された。該クローンより、本発明のDNAを各々取得した。

【0193】〔実施例4〕本発明のDNAの各種臓器における発現量の検出

COL03279、COL06772、ADKA01604、ADSU00701の各クローンに認められる本発明のDNAの各種臓器における発現量の定量を、定法（PCR Protocols, Academic Press (1990)等）に従い、半定量的PCR法を用い、以下のように行った。また、どの細胞でも同程度発現していると考えられるグリセルアルデヒド－3－リン酸デヒドロゲナーゼ（glyceraldehyde-3-phosphate dehydrogenase:G3PDH）の転写産物の定量を同時に行ない、細胞間でのmRNA量の違いや、サンプル間での逆転写酵素によるmRNAから一本鎖cDNAへの変換効率に大差ないことを確認した。

【0194】ヒト臓器由来のmRNA（Clontech社製：1副腎、2脳、3尾状核、4海馬、5黒質、6視床、7腎臓、8脾臓、9脳下垂体、10小腸、11骨髄、12扁桃体、13小脳、14脳梁、15胎児脳、16胎児腎臓、17胎児肝臓、18胎児肺、19心臓、20肝臓、21肺、22リンパ節、23乳腺、24胎盤、25前立腺、26唾液腺、27骨格筋、28脊髄、29脾臓、30胃、31精巣、32胸腺、33甲状腺、34気管、35子宮）からcDNA合成キット（SUPERSCRIPT™ Preamplification System: BRL社製）を用いて、一本鎖cDNAを合成した。1μgのmRNAから一本鎖cDNAを合成し、水で240倍希釈してPCRの鋳型として使

用した。ＰＣＲ用プライマーとしては、ＣＯＬ０３２７９からの塩基配列情報に基づいた配列番号１６および１７、ＣＯＬ０６７７２からの塩基配列情報に基づいた配列番号１８および１９、ＡＤＫＡ０１６０４からの塩基配列情報に基づいた配列番号２０および２１、ＡＤＳＵ００７０１からの塩基配列情報に基づいた配列番号２２および２３に記載の合成ＤＮＡを用いた。ＰＣＲ反応は、ニッポンジーン社製のRecombinant Taq DNA Polymerase (GeneTaq) と添付の１０×Gene Taq Universal Bufferおよび2．５ mmol/ldＮＴＰ Ｍｉｘｔｕｒｅを用いて、説明書に従って行った。ＭＪ ＲＥＳＥＲＣＨ社製のサーマル・サイクラーを用いて、９４℃で３０秒間、６０℃で１分間、７２℃で２分間の反応を２６～３０サイクル行った。反応液をアガロースゲル電気泳動法およびエチジウムブロマイド染色により解析した。

【０１９５】結果を図１～４に示す。ＣＯＬ０３２７９、ＣＯＬ０６７７２、ＡＤＫＡ０１６０４、ＡＤＳＵ００７０１の各クローンに認められる本発明のＤＮＡは、各クローン、各臓器によって強弱の差はあるものの、検討した35種全ての臓器で発現していた。

【０１９６】

【発明の効果】本発明によれば、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エンドトキシンショック、敗血症、微生物感染、慢性Ｂ型肝炎、慢性Ｃ型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、外傷性脳損傷、乾癬、痛風、各種脳脊髄炎、うっ血性心不全、炎症性腸疾患等の感染や炎症を伴う疾患、バーキットリンパ腫、ホジキン病、各種リンパ腫、成人Ｔ細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、慢性関節リウマチ、変形性関節炎等の異常な線維芽細胞や滑膜組織の活性化を伴う疾患、エイズ等のウイルス性疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄等の平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群（ＳＩＲＳ：syste mic inflammatory responsesyndrome）、成人呼吸窮迫症候群（ＡＲＤＳ：adult respiratory distress syndrome）等の治療薬の探索、開発に有用なポリペプチド、該ポリペプチドをコードするＤＮＡ、該ＤＮＡのアンチセンスＤＮＡ／ＲＮＡ、該ＤＮＡを用いた遺伝子治療、該ポリペプチドを認識する抗体、該ポリペプチドの活性上昇改変体、該ポリペプチドのドミナントネガティブ変異体、およびこれらの利用法を提供することができる。

【０１９７】

【配列表フリーテキスト】

配列番号１１－人工配列の説明：合成RNA（オリゴキャップリンカー配列）

配列番号１２－人工配列の説明：合成DNA（オリゴdTプライマー配列）

配列番号１３－人工配列の説明：合成DNA（5'末端側のセンスプライマー配列）

配列番号１４－人工配列の説明：合成DNA（3'末端側のアンチセンスプライマー配列）

配列番号１５－人工配列の説明（転写因子NF-κ結合配列）

配列番号１６－人工配列の説明：合成DNA（組織発現分布を検討した合成プライマー配列）

配列番号１７－人工配列の説明：合成DNA

配列番号１８－人工配列の説明：合成DNA

配列番号１９－人工配列の説明：合成DNA

配列番号２０－人工配列の説明：合成DNA

配列番号２１－人工配列の説明：合成DNA

配列番号２２－人工配列の説明：合成DNA

配列番号２３－人工配列の説明：合成DNA

【０１９８】

【配列表】

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<120> Novel polypeptide

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【０１９９】

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【0200】

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【0201】

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65 70 75 80
Thr Ala Gly Ile Phe Asp Ala Tyr Val Pro Pro Glu Gly Asp Ala Arg
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Ile Ser Ser Leu Ser Lys Glu Gly Leu Ile Glu Arg Thr Glu Arg Met
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His Thr Leu Val Thr Glu His Cys Phe Pro Asp Met Thr Trp Asp Ile
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Lys Tyr Lys Thr Val Arg Trp Ser Phe Val Glu Ser Leu Glu Pro Ser
            180                 185                 190
His Val Val Gln Val Arg Cys Ser Ser Met Met Asn Gln Gly Asn Val
        195                 200                 205
Tyr Gly Gln Ile Thr Val Arg Met His Thr Arg Gln Thr Leu Ala Ile
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Tyr Asp Arg Phe Gly Arg Leu Met Tyr Gly Gln Glu Asp Val Pro Lys
225                 230                 235                 240
Asp Val Leu Glu Tyr Val Val Phe Glu Lys Gln Leu Thr Asn Pro Tyr
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Gly Ser Trp Arg Met His Thr Lys Ile Val Pro Pro Trp Ala Pro Pro
            260                 265                 270
Lys Gln Pro Ile Leu Lys Thr Val Met Ile Pro Gly Pro Gln Leu Lys
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        35                  40                  45
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    50                  55                  60
Arg Arg Asn Thr Ala Pro Val Arg Arg Ile Glu His Leu Gly Ser Thr
65                  70                  75                  80
Lys Ser Leu Asn His Ser Lys Gln Arg Ser Thr Leu Pro Arg Ser Phe
                85                  90                  95
Ser Leu Asp Pro Leu Met Glu Arg Arg Trp Asp Leu Asp Leu Thr Tyr
            100                 105                 110
Val Thr Glu Arg Ile Leu Ala Ala Ala Phe Pro Ala Arg Pro Asp Glu
        115                 120                 125
Gln Arg His Arg Gly His Leu Arg Glu Leu Ala His Val Leu Gln Ser
    130                 135                 140
Lys His Arg Asp Lys Tyr Leu Leu Phe Asn Leu Ser Glu Lys Arg His
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Asp Leu Thr Arg Leu Asn Pro Lys Val Gln Asp Phe Gly Trp Pro Glu
                165                 170                 175
Leu His Ala Pro Pro Leu Asp Lys Leu Cys Ser Ile Cys Lys Ala Met
            180                 185                 190
Glu Thr Trp Leu Ser Ala Asp Pro Gln His Val Val Val Leu Tyr Cys
        195                 200                 205
Lys Val Gly Gln Asp Leu Gly Phe Pro Gly Ala Trp Arg Phe Gln Val
    210                 215                 220
Ser Leu Glu Leu Pro Asp Pro His Pro Cys Leu Ser Val Cys Gln Gly
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【0203】

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Glu Glu Cys Gln Leu Gln Leu Lys Thr Leu His Glu Asp Leu Ser Gly
        35                  40                  45
Arg Leu Glu Glu Ser Leu Ser Ile Ile Asn Glu Lys Val Pro Phe Asn
    50                  55                  60
Asp Thr Lys Tyr Ser Arg Tyr Asn Ala Leu Asn Val Pro Leu His Asn
65                  70                  75                  80
Arg Arg His Gln Leu Lys Met Arg Asp Ile Ala Gly Gln Ala Leu Ala
                85                  90                  95
Phe Val Gln Asp Leu Val Thr Ala Leu Leu Asn Phe His Thr Tyr Thr
            100                 105                 110
Glu Gln Arg Ile Gln Ile Phe Pro Val Asp Ser Ala Ile Asp Thr Ile
        115                 120                 125
Ser Pro Leu Asn Gln Lys Phe Ser Gln Tyr Leu His Glu Asn Ala Ser
    130                 135                 140
Tyr Val Arg Pro Leu Glu Glu Gly Met Leu His Leu Phe Glu Ser Ile
145                 150                 155                 160
Thr Glu Asp Thr Val Thr Val Leu Glu Thr Thr Val Lys Leu Lys Thr
                165                 170                 175
Phe Ser Glu His Leu Thr Ser Tyr Ile Cys Phe Leu Arg Lys Ile Leu
            180                 185                 190
Pro Tyr Gln Leu Lys Ser Leu Glu Glu Glu Cys Glu Ser Ser Leu Cys
        195                 200                 205
Thr Ser Ala Leu Arg Ala Arg Asn Leu Glu Leu Ser Gln Asp Met Lys
    210                 215                 220
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Lys Met Thr Ala Val Phe Glu Lys Leu Gln Thr Tyr Ile Ala Leu Leu
225 230 235 240
Ala Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu Arg Thr Asn Tyr Ser
245 250 255
Ser Val Leu Thr Asn Val Gly Ala Ala Leu His Gly Phe His Asp Val
260 265 270
Met Lys Asp Ile Ser Lys His Tyr Ser Gln Lys Ala Ala Ile Glu His
275 280 285
Glu Leu Pro Thr Ala Thr Gln Lys Leu Ile Thr Thr Asn Asp Cys Ile
290 295 300
Leu Ser Ser Val Val Ala Ser Thr Asn Gly Ala Gly Lys Ile Ala Ser
305 310 315 320
Phe Phe Ser Asn Asn Leu Asp Tyr Phe Ile Ala Ser Leu Ser Tyr Gly
325 330 335
Pro Lys Ala Ala Ser Gly Phe Ile Ser Pro Leu Ser Ala Glu Cys Met
340 345 350
Leu Gln Tyr Lys Lys Lys Ala Ala Ala Tyr Met Lys Ser Leu Arg Lys
355 360 365
Pro Leu Leu Glu S er Val Pro Tyr Glu Glu Ala Leu Ala Asn Arg Arg
370 375 380
Ile Leu Leu Ser Ser Thr Glu Ser Arg Glu Gly Leu Ala Gln Gln Val
385 390 395 400
Gln Gln Ser Leu Glu Lys Ile Ser Lys Leu Glu Gln Glu Lys Glu His
405 410 415
Trp Met Leu Glu Ala Gln Leu Ala Lys Ile Lys Leu Glu Lys Glu Asn
420 425 430
Gln Arg Ile Ala Asp Lys Leu Lys Asn Thr Gly Ser Ala Gln Leu Val
435 440 445
Gly Leu Ala Gln Glu Asn Ala Ala Val Ser Asn Thr Ala Gly Gln Asp
450 455 460
Glu Ala Thr Ala Lys Ala Val Leu Glu Pro Ile Gln Ser Thr Ser Leu
465 470 475 480
Ile Gly Thr Leu Thr Arg Thr Ser Asp Ser Glu Val Pro Asp Val Glu
485 490 495
Ser Arg Glu Asp Leu Ile Lys Asn Arg Tyr Met Ala Arg Ile Val Glu
500 505 510
Leu Thr Ser Gln Leu Gln Leu Ala Asp Ser Lys Ser Val His Phe Tyr
515 520 525
Ala Glu Cys Arg Ala Leu Ser Lys Arg Leu Ala Leu Ala Glu Lys Ser
530 535 540
Lys Glu Ala Leu Thr Glu Glu Met Lys Leu Ala Ser Gln Asn Ile Ser
545 550 555 560
Arg Leu Gln Asp Glu Leu Thr Thr Thr Lys Arg Ser Tyr Glu Asp Gln
565 570 575
Leu Ser Met Met Ser Asp His Leu Cys Ser Met Asn Glu Thr Leu Ser
580 585 590
Lys Gln Arg Glu Glu Ile Asp Thr Leu Lys Met Ser Ser Lys Gly Asn
595 600 605
Ser Lys Lys Asn Lys Ser Arg

610

【0204】

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<213> Homo sapiens
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<222> (158)..(2497)
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tactgcctga cccgttcccg ggagcgtgtc tgggtttggg ggcgggagac aggctgagcc 120
gcctgggcgg cctggcctgt acggggcggg ggaggcc atg gcc tcg gct gag ttg 175
Met Ala Ser Ala Glu Leu
1 5

cag ggg aag tac cag aag ctg gct cag gag tac tcg aag ctt cgg gct 223
Gln Gly Lys Tyr Gln Lys Leu Ala Gln Glu Tyr Ser Lys Leu Arg Ala
10 15 20

cag aat cag gtt ctg aaa aag ggt gtt gtg gat gaa caa gca aat tct 271
Gln Asn Gln Val Leu Lys Lys Gly Val Val Asp Glu Gln Ala Asn Ser
25 30 35

gca gct tta aag gag caa ctg aaa atg aag gat cag tca ttg aga aaa 319
Ala Ala Leu Lys Glu Gln Leu Lys Met Lys Asp Gln Ser Leu Arg Lys
40 45 50

cta caa cag gaa atg gac agt ttg aca ttt cga aat ctg cag ctt gcc 367
Leu Gln Gln Glu Met Asp Ser Leu Thr Phe Arg Asn Leu Gln Leu Ala
55 60 65 70

aag agg gta gaa cta ctt caa gat gaa cta gct cta agt gaa cca cga 415
Lys Arg Val Glu Leu Leu Gln Asp Glu Leu Ala Leu Ser Glu Pro Arg
75 80 85

ggc aag aaa aac aag aaa agt gga gaa tct tct tct cag ttg agt caa 463
Gly Lys Lys Asn Lys Lys Ser Gly Glu Ser Ser Ser Gln Leu Ser Gln
90 95 100

gag cag aag agt gtc ttt gat gaa gat ctg caa aag aag ata gaa gag 511
Glu Gln Lys Ser Val Phe Asp Glu Asp Leu Gln Lys Lys Ile Glu Glu
105 110 115

aat gaa cgg ttg cat ata caa ttt ttt gaa gct gat gag cag cac aag 559
Asn Glu Arg Leu His Ile Gln Phe Phe Glu Ala Asp Glu Gln His Lys
120 125 130

cat gtg gaa gca gag ctg agg agt cga ctg gcc act ctg gag aca gaa 607
His Val Glu Ala Glu Leu Arg Ser Arg Leu Ala Thr Leu Glu Thr Glu
135 140 145 150

gca gcc cag cac caa gct gtg gtt gac ggt ctc acc cgg aag tac atg 655
Ala Ala Gln His Gln Ala Val Val Asp Gly Leu Thr Arg Lys Tyr Met
155 160 165

gaa acc att gag aag ctg cag aac gac aag gct aaa cta gaa gtg aaa 703
Glu Thr Ile Glu Lys Leu Gln Asn Asp Lys Ala Lys Leu Glu Val Lys
170 175 180

tct cag act cta gaa aag gaa gcc aag gaa tgt cga ctt cga acg gaa 751

71 72

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Ser Gln Thr Leu Glu Lys Glu Ala Lys Glu Cys Arg Leu Arg Thr Glu
        185                 190                 195
gaa tgt caa tta cag tta aag act ctt cat gaa gat ttg tca ggt aga   799
Glu Cys Gln Leu Gln Leu Lys Thr Leu His Glu Asp Leu Ser Gly Arg
    200                 205                 210
tta gag gaa tcc tta tca atc atc aat gaa aaa gta cct ttt aat gat   847
Leu Glu Glu Ser Leu Ser Ile Ile Asn Glu Lys Val Pro Phe Asn Asp
215                 220                 225                 230
aca aaa tat agt cag tac aac gct ctg aac gtt cca ctc cac aat agg   895
Thr Lys Tyr Ser Gln Tyr Asn Ala Leu Asn Val Pro Leu His Asn Arg
                235                 240                 245
aga cac cag ctg aag atg cga gat att gct ggg cag gcc ctg gct ttt   943
Arg His Gln Leu Lys Met Arg Asp Ile Ala Gly Gln Ala Leu Ala Phe
            250                 255                 260
gtt cag gat ctt gtg acg gct ctt cta aac ttt cat acc tac aca gaa   991
Val Gln Asp Leu Val Thr Ala Leu Leu Asn Phe His Thr Tyr Thr Glu
        265                 270                 275
cag agg att caa att ttt cct gtt gat tct gcc att gac act ata tct  1039
Gln Arg Ile Gln Ile Phe Pro Val Asp Ser Ala Ile Asp Thr Ile Ser
    280                 285                 290
cca ttg aat cag aag ttc tca caa tac ctt cat gaa aat gcg tcc tat  1087
Pro Leu Asn Gln Lys Phe Ser Gln Tyr Leu His Glu Asn Ala Ser Tyr
295                 300                 305                 310
gtc cgc cct ctt gag gaa gga atg ctt cat tta ttt gaa agt atc act  1135
Val Arg Pro Leu Glu Glu Gly Met Leu His Leu Phe Glu Ser Ile Thr
                315                 320                 325
gag gat act gtg act gtc ttg gag aca act gtg aaa ttg aaa act ttt  1183
Glu Asp Thr Val Thr Val Leu Glu Thr Thr Val Lys Leu Lys Thr Phe
            330                 335                 340
tca gaa cac tta acc tcc tac ata tgt ttt ctt agg aag att ctt ccc  1231
Ser Glu His Leu Thr Ser Tyr Ile Cys Phe Leu Arg Lys Ile Leu Pro
        345                 350                 355
tat cag tta aaa agt tta gaa gaa gaa tgt gaa tcc tct ctt tgc aca  1279
Tyr Gln Leu Lys Ser Leu Glu Glu Glu Cys Glu Ser Ser Leu Cys Thr
    360                 365                 370
tct gcg tta aga gcc agg aat cta gag ctg tcc cag gac atg aaa aaa  1327
Ser Ala Leu Arg Ala Arg Asn Leu Glu Leu Ser Gln Asp Met Lys Lys
375                 380                 385                 390
atg aca gct gtg ttt gag aag ctg cag act tac ata gct ctt ctt gcc  1375
Met Thr Ala Val Phe Glu Lys Leu Gln Thr Tyr Ile Ala Leu Leu Ala
                395                 400                 405
ttg cca agt aca gag cca gat gga ctc ctt cgg aca aac tac agt tct  1423
Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu Arg Thr Asn Tyr Ser Ser
            410                 415                 420
gtg tta aca aat gtt ggt gct gct ctg cat gga ttt cat gac gtt atg  1471
Val Leu Thr Asn Val Gly Ala Ala Leu His Gly Phe His Asp Val Met
        425                 430                 435
aaa gat att tcc aaa cat tat agt caa aaa gct gca ata gag cat gaa  1519
Lys Asp Ile Ser Lys His Tyr Ser Gln Lys Ala Ala Ile Glu His Glu
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73 74

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    440                 445                 450
ctt cca aca gca aca cag aag ctg ata aca act aat gac tgt atc ctg  1567
Leu Pro Thr Ala Thr Gln Lys Leu Ile Thr Thr Asn Asp Cys Ile Leu
455                 460                 465                 470
tca tca gta gtg gca tta aca aat gga gca gga aag att gca tcc ttc  1615
Ser Ser Val Val Ala Leu Thr Asn Gly Ala Gly Lys Ile Ala Ser Phe
                475                 480                 485
ttc agc aac aat ttg gac tac ttc att gct tca ctg agc tat gga cct  1663
Phe Ser Asn Asn Leu Asp Tyr Phe Ile Ala Ser Leu Ser Tyr Gly Pro
            490                 495                 500
aag gca gcg agt gga ttc att agt cct ctt tca gct gaa tgc atg cta  1711
Lys Ala Ala Ser Gly Phe Ile Ser Pro Leu Ser Ala Glu Cys Met Leu
        505                 510                 515
cag tat aag aaa aaa gct gct gcc tat atg aag tct ttg aga aag ccc  1759
Gln Tyr Lys Lys Lys Ala Ala Ala Tyr Met Lys Ser Leu Arg Lys Pro
    520                 525                 530
ctc ttg gag tct gtg cct tat gaa gaa gca ctg gca aac cgc cgc atc  1807
Leu Leu Glu Ser Val Pro Tyr Glu Glu Ala Leu Ala Asn Arg Arg Ile
535                 540                 545                 550
ctt ctc agc tct act gaa agt cga gaa ggc ctt gca cag caa gtt caa  1855
Leu Leu Ser Ser Thr Glu Ser Arg Glu Gly Leu Ala Gln Gln Val Gln
                555                 560                 565
cag agt ttg gaa aag att tct aaa ctg gag cag gaa aaa gaa cat tgg  1903
Gln Ser Leu Glu Lys Ile Ser Lys Leu Glu Gln Glu Lys Glu His Trp
            570                 575                 580
atg ttg gaa gca caa tta gcc aaa atc aag cta gag aaa gaa aac cag  1951
Met Leu Glu Ala Gln Leu Ala Lys Ile Lys Leu Glu Lys Glu Asn Gln
        585                 590                 595
cga att gca gat aag ctg aag aat aca ggt agt gcc cag ctg gtt ggg  1999
Arg Ile Ala Asp Lys Leu Lys Asn Thr Gly Ser Ala Gln Leu Val Gly
    600                 605                 610
ctg gcc cag gaa aat gct gct gtg tca aat act gct ggc cag gat gaa  2047
Leu Ala Gln Glu Asn Ala Ala Val Ser Asn Thr Ala Gly Gln Asp Glu
615                 620                 625                 630
gcc aca gct aag gct gtg ttg gag ccc att cag agc acc agt cta att  2095
Ala Thr Ala Lys Ala Val Leu Glu Pro Ile Gln Ser Thr Ser Leu Ile
                635                 640                 645
ggg act tta acc agg aca tct gac agt gag gtt cca gat gtg gaa tct  2143
Gly Thr Leu Thr Arg Thr Ser Asp Ser Glu Val Pro Asp Val Glu Ser
            650                 655                 660
cgt gaa gac tta att aaa aat cac tac atg gca agg ata gtg gaa ctt  2191
Arg Glu Asp Leu Ile Lys Asn His Tyr Met Ala Arg Ile Val Glu Leu
        665                 670                 675
acg tct cag ttg cag ctg gct gac agt aag tca gtg cat ttt tat gcc  2239
Thr Ser Gln Leu Gln Leu Ala Asp Ser Lys Ser Val His Phe Tyr Ala
    680                 685                 690
gag tgc cga gca ctg tct aaa aga ctg gcc ttg gct gaa aag tct aag  2287
Glu Cys Arg Ala Leu Ser Lys Arg Leu Ala Leu Ala Glu Lys Ser Lys
695                 700                 705                 710
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gaa gca ttg aca gaa gaa atg aaa ctt gcc agt cag aac atc agc aga 2335
Glu Ala Leu Thr Glu Glu Met Lys Leu Ala Ser Gln Asn Ile Ser Arg
715 720 725
ctt cag gat gag ctg aca act acc aag agg agt tac gag gat cag tta 2383
Leu Gln Asp Glu Leu Thr Thr Thr Lys Arg Ser Tyr Glu Asp Gln Leu
730 735 740
agt atg atg agt gac cac ctg tgc agc atg aat gag aca tta tct aaa 2431
Ser Met Met Ser Asp His Leu Cys Ser Met Asn Glu Thr Leu Ser Lys

745 750 755
cag aga gaa gag att gac aca cta aag atg tcc agt aag ggg aat tct 2479
Gln Arg Glu Glu Ile Asp Thr Leu Lys Met Ser Ser Lys Gly Asn Ser
760 765 770
aaa aag aac aag agt cga tagttttgaa atagctggtt ggcgactgtt 2527
Lys Lys Asn Lys Ser Arg
775 780
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gccttcagga agctaaagta ttgttggacc tagtaaacta gtcagtgttg gaaacggcct 2647
tgaaatattt aaaacatatt tgtaaccagt gaggcaaata cagaagttga tgtcggcagt 2707
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tatcattatc ttcagctctt tgataccctg tgttagagta atagctaaag gaagttcatg 3127
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【0205】

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<222> (49)..(507)
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Met Leu Lys
1
gcc agt gcc gcg tcc cct gct gtt gcc ctt aaa gca ctt gag gtc cag 105
Ala Ser Ala Ala Ser Pro Ala Val Ala Leu Lys Ala Leu Glu Val Gln
5 10 15
att gtt gag gag gcg act cag aac gcg gag gag cag ccg agt act ttc 153
Ile Val Glu Glu Ala Thr Gln Asn Ala Glu Glu Gln Pro Ser Thr Phe
20 25 30 35
agc gaa aat gag tat gat gcc agt tgg tcc cca tca tgg gtc atg tgg 201
Ser Glu Asn Glu Tyr Asp Ala Ser Trp Ser Pro Ser Trp Val Met Trp
40 45 50
ctt ggg ctt ccc agc aca ctt cat agc tgc cac gat ata gtt tta cga 249

Leu Gly Leu Pro Ser Thr Leu His Ser Cys His Asp Ile Val Leu Arg
55 60 65
aga agt tac ttg gga agt tgg ggc ttt agt atc gtt ggt gga tat gaa 297
Arg Ser Tyr Leu Gly Ser Trp Gly Phe Ser Ile Val Gly Gly Tyr Glu
70 75 80
gag aac cac acc aat cag cct ttt ttc att aaa act att gtc ttg gga 345
Glu Asn His Thr Asn Gln Pro Phe Phe Ile Lys Thr Ile Val Leu Gly
85 90 95
act cct gct tat tat gat gga aga tta aag tgt ggt gac atg att gtg 393
Thr Pro Ala Tyr Tyr Asp Gly Arg Leu Lys Cys Gly Asp Met Ile Val
100 105 110 115
gcc gta aat ggg ctg tca acc gtg ggc atg agc cac tct gca cta gtt 441
Ala Val Asn Gly Leu Ser Thr Val Gly Met Ser His Ser Ala Leu Val
120 125 130
ccc atg ttg aag gag cag agg aac aaa gtc act ctg acc gtt att tgt 489
Pro Met Leu Lys Glu Gln Arg Asn Lys Val Thr Leu Thr Val Ile Cys
135 140 145
tgg cct ggc agc ctt gta t agattttgg aaattggttt caaatcttgc 537
Trp Pro Gly Ser Leu Val
150
atcttccttt tttagatttt tgaaagaaaa ccctttggtt tcattgtgtt tgtggtttag 597
gagctgctga cactgctggt atacacaggg ccaaaaccca ctaagattgt ccgtttatgt 657
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aatgagcaaa gctcacccaa actgtgcccc agatggagta aagaccttct ggtgggtctt 837
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gtttctcatc tgaaaagtag catactaaca cacagctttt aaaaacttta tactttttgtt 1617
tttttgtttt tttttaagac ggagtctggc tctgtttccc aggttgcagt gagcagagat 1677
cgtgccactg cactctagcc ttggtgacag agcaagactc tgtgtcaaaa aaaaaaaaaa 1737
aaa 1740

【0206】

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<211> 1574
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<213> Homo sapiens
<220>
<221> CDS

79 80

<222> (22)..(939)
<400> 8

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                        Met Ala Ala Pro Ile Pro Gln Gly Phe Ser
                          1               5                  10
tgt tta tcg agg ttt ttg ggc tgg tgg ttt cgg cag cca gtt ctg gtg   99
Cys Leu Ser Arg Phe Leu Gly Trp Trp Phe Arg Gln Pro Val Leu Val
                 15                  20                  25
act cag tcc gca gct ata gtt cca gta aga act aaa aaa cgt ttc aca  147
Thr Gln Ser Ala Ala Ile Val Pro Val Arg Thr Lys Lys Arg Phe Thr
             30                  35                  40
cct cct att tat caa cct aaa ttt aaa aca gaa aag gag ttt atg caa  195
Pro Pro Ile Tyr Gln Pro Lys Phe Lys Thr Glu Lys Glu Phe Met Gln
         45                  50                  55
cat gcc cgg aaa gca gga ttg gtt att cct cca gaa aaa tcg gac cgt  243
His Ala Arg Lys Ala Gly Leu Val Ile Pro Pro Glu Lys Ser Asp Arg
     60                  65                  70
tcc ata cat ctg gcc tgt aca gct ggt ata ttt gat gcc tat gtt cct  291
Ser Ile His Leu Ala Cys Thr Ala Gly Ile Phe Asp Ala Tyr Val Pro
 75                  80                  85                  90
cct gag ggt gat gca cgc ata tca tct ctt tca aag gag gga ctg ata  339
Pro Glu Gly Asp Ala Arg Ile Ser Ser Leu Ser Lys Glu Gly Leu Ile
                 95                 100                 105
gag aga act gaa cga atg aag aag act atg gca tca caa gtg tca atc  387
Glu Arg Thr Glu Arg Met Lys Lys Thr Met Ala Ser Gln Val Ser Ile
            110                 115                 120
cgg agg ata aaa gac tat gat gcc aac ttt aaa ata aag gac ttc cct  435
Arg Arg Ile Lys Asp Tyr Asp Ala Asn Phe Lys Ile Lys Asp Phe Pro
        125                 130                 135
gga aaa gct aag gat atc ttt att gaa gct cac ctt tgt cta aat aac  483
Gly Lys Ala Lys Asp Ile Phe Ile Glu Ala His Leu Cys Leu Asn Asn
    140                 145                 150
tca gac cat gac cga ctt cat acc ttg gta act gaa cac tgt ttt cca  531
Ser Asp His Asp Arg Leu His Thr Leu Val Thr Glu His Cys Phe Pro
155                 160                 165                 170
gac atg act tgg gac atc aaa tat aag acc gtc cgc tgg agc ttt gtg  579
Asp Met Thr Trp Asp Ile Lys Tyr Lys Thr Val Arg Trp Ser Phe Val
                175                 180                 185
gaa tct tta gag ccc tct cat gtt gtt caa gtt cgc tgt tca ag t atg  627
Glu Ser Leu Glu Pro Ser His Val Val Gln Val Arg Cys Ser Ser Met
            190                 195                 200
atg aac cag ggc aac gtg tac ggc cag atc acc gta cgc atg cac acc  675
Met Asn Gln Gly Asn Val Tyr Gly Gln Ile Thr Val Arg Met His Thr
        205                 210                 215
cgg cag act ctg gcc atc tat gac cgg ttt ggc cgg ttg atg tat gga  723
Arg Gln Thr Leu Ala Ile Tyr Asp Arg Phe Gly Arg Leu Met Tyr Gly
    220                 225                 230
cag gaa gat gta ccc aag gat gtc ctg gag tat gtt gta ttc gaa aag  771
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81 82

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Gln Glu Asp Val Pro Lys Asp Val Leu Glu Tyr Val Val Phe Glu Lys
235                 240                 245                 250
cag ttg aca aac ccc tat gga agc tgg aga atg cat acc aag atc gtt  819
Gln Leu Thr Asn Pro Tyr Gly Ser Trp Arg Met His Thr Lys Ile Val
                255                 260                 265
ccc cca tgg gca ccc cct aag cag ccc atc ctt aag acg gtg atg atc  867
Pro Pro Trp Ala Pro Pro Lys Gln Pro Ile Leu Lys Thr Val Met Ile
            270                 275                 280
cct ggc cct cag ctg aaa cca gaa gaa gaa tat gaa gag gca caa gga  915
Pro Gly Pro Gln Leu Lys Pro Glu Glu Glu Tyr Glu Glu Ala Gln Gly
        285                 290                 295
gag gcc cag aag cct cag cta gcc tgatgacaaa aatgacttct agggtgaagc  969
Glu Ala Gln Lys Pro Gln Leu Ala
    300                 305
ctgggtgatg aggctgctgg aagctttgaa gtctcccatt cccctcatgc tataaaaaga 1029
actacctttg ttctctccca tcctgctcag gtcttttcag cagtctcatc atcagcaacc 1089
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aataattagt gtttataaga catttaagag gccctttttc atatactgac tcactgatga 1509
atcagcattt gcattttatg gaaaaatata aatgcaaaga aataatttaa aaaaaaaaaa 1569
aaaaa                                                            1574
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【0207】

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<212> DNA
<213> Homo sapiens
<220>
<221> CDS
<222> (55)..(837)
<400> 9

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aag cct agg aaa gct gag cct cat agc ttc cgg gag aag gtt ttc cgg  105
Lys Pro Arg Lys Ala Glu Pro His Ser Phe Arg Glu Lys Val Phe Arg
          5                  10                  15
aag aaa cct cca gtc tgt gca gta tgt aag gtg acc atc gat ggg aca  153
Lys Lys Pro Pro Val Cys Ala Val Cys Lys Val Thr Ile Asp Gly Thr
         20                  25                  30
ggc gtt tcg tgc aga gtc tgc aag gtg gcg acg cac aga aaa tgc gaa  201
Gly Val Ser Cys Arg Val Cys Lys Val Ala Thr His Arg Lys Cys Glu
     35                  40                  45
gca aag gtg act tca gcc tgt cag gcc ttg cct ccc gtg gag ttg cgg  249
Ala Lys Val Thr Ser Ala Cys Gln Ala Leu Pro Pro Val Glu Leu Arg
 50                  55                  60                  65
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83 84

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cga aac acg gcc cca gtc agg cgc ata gag cac ctg gga tcc acc aaa  297
Arg Asn Thr Ala Pro Val Arg Arg Ile Glu His Leu Gly Ser Thr Lys
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Ser Leu Asn His Ser Lys Gln Arg Ser Thr Leu Pro Arg Ser Phe Ser
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Leu Asp Pro Leu Met Glu Arg Arg Trp Asp Leu Asp Leu Thr Tyr Val
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Thr Glu Arg Ile Leu Ala Ala Ala Phe Pro Ala Arg Pro Asp Glu Gln
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Arg His Arg Gly His Leu Arg Glu Leu Ala His Val Leu Gln Ser Lys
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His Arg Asp Lys Tyr Leu Leu Phe Asn Leu Ser Glu Lys Arg His Asp
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His Ala Pro Pro Leu Asp Lys Leu Cys Ser Ile Cys Lys Ala Met Glu
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Val Gly Gln Asp Leu Gly Phe Pro Gly Ala Trp Arg Phe Gln Val Ser
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Lys Leu Gln Asn Asp Lys Ala Lys Leu Glu Val Lys Ser Gln Thr Leu
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Glu Lys Glu Ala Lys Glu Cys Arg Leu Arg Thr Glu Glu Cys Gln Leu
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Gln Leu Lys Thr Leu His Glu Asp Leu Ser Gly Arg Leu Glu Glu Ser
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Arg Tyr Asn Ala Leu Asn Val Pro Leu His Asn Arg Arg His Gln Leu
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Ala Arg Asn Leu Glu Leu Ser Gln Asp Met Lys Lys Met Thr Ala Val
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Phe Glu Lys Leu Gln Thr Tyr Ile Ala Leu Leu Ala Leu Pro Ser Thr
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【図面の簡単な説明】

【図１】は、ＰＣＲ法を用いて、35種のヒト組織（臓器）におけるＣＯＬ０３２７９転写物の発現量を調べた結果である。

【図２】は、ＰＣＲ法を用いて、35種のヒト組織（臓器）におけるＣＯＬ０６７７２転写物の発現量を調べた結果である。

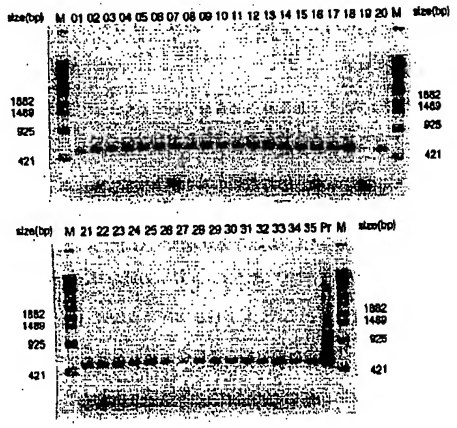
【図３】は、ＰＣＲ法を用いて、35種のヒト組織（臓器）におけるＡＤＫＡ０１６０４転写物の発現量を調べた結果である。

【図４】は、ＰＣＲ法を用いて、35種のヒト組織（臓器）におけるＡＤＳＵ００７０１転写物の発現量を調べた結果である。

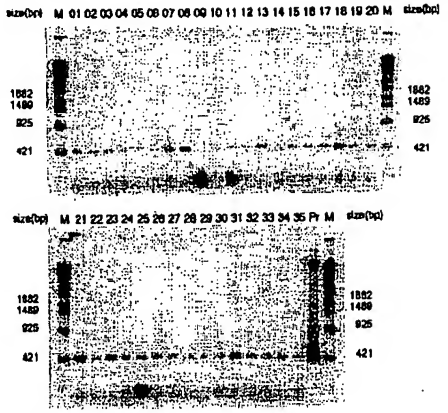
【符号の説明】

全図中に記載の数字、英字は以下の通りである。
０１：副腎、０２：脳、０３：尾状核、０４：海馬、０５：黒質、０６：視床、０７：腎臓、０８：脾臓、０９脳下垂体、１０：小腸、１１：骨髄、１２：扁桃体、１３：小脳、１４：脳梁、１５：胎児脳、１６：胎児腎臓、１７：胎児肝臓、１８：胎児肺、１９：心臓、２０：肝臓、２１：肺、２２：リンパ節、２３：乳腺、２４：胎盤、２５：前立腺、２６：唾液腺、２７：骨格筋、２８：脊髄、２９：脾臓、３０：胃、３１：精巣、３２：胸腺、３３：甲状腺、３４：気管、３５：子宮、Ｐｒ：プラスミド、Ｍ：分子量マーカー

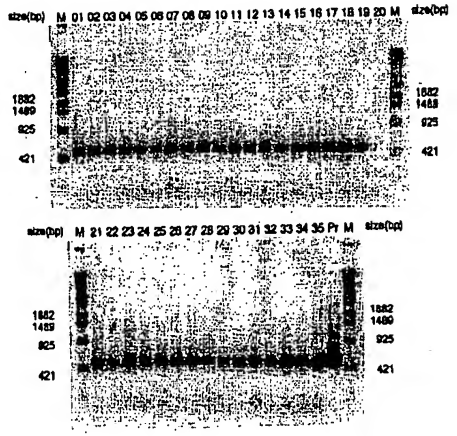
【図１】



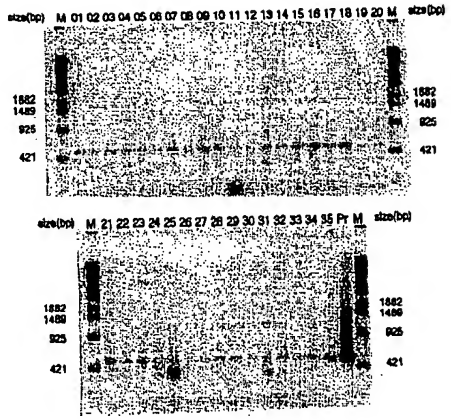
【図２】



【図３】



【図４】



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(51)Int.Cl.[7]	識別記号	FI		テーマコード(参考)
A61K 39/395		A61K	48/00	4B065
		A61P	3/10	4C084
48/00			9/04	4C085
A61P 3/10			9/10	4H045
9/04			11/00	
9/10			11/02	
11/00			11/04	
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37/08		C07K	14/47	
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C07K 14/47		C12N	1/21	
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東京都杉並区南荻窪４－８－13

Ｆターム(参考) 2B030 AB03 AD05 CA06 CA17 CA19
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4C085 AA11 DD62
4H045 AA10 AA11 AA20 AA30 BA10 CA40 DA75 EA20 EA50 FA71 FA74

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2001-352986
(43)Date of publication of application : 25.12.2001

(51)Int.Cl.

C12N 15/09
A01H 5/00
A01K 67/027
A01K 67/033
A61K 38/00
A61K 39/395
A61K 48/00
A61P 3/10
A61P 9/04
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(C12N 5/10
C12R 1:91)

(21)Application number : 2000-175475
(22)Date of filing : 12.06.2000

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(72)Inventor : OBATA CHOEI
NISHI TATSUYA
OTA NORIO
NAKAMURA YUSUKE
SUGANO SUMIO

(54) NEW POLYPEPTIDE

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a polypeptide useful for screening for and/or developing an agent for treating, preventing, and/or diagnosing a disease related to the activation of NF-κB, a DNA encoding the polypeptide, an antisense DNA/RNA of the DNA the gene therapy using the DNA, an antibody recognizing the polypeptide, a modified polypeptide derived from the preceding polypeptide and having an enhanced activity, a dominant negative variant of the polypeptide, and methods for utilizing these.
SOLUTION: A polypeptide activating NF-κB is identified to produce a DNA encoding the polypeptide and an antibody recognizing the polypeptide. These can be utilized fro screening for a medicine for and diagnosing a disease related to the activation of NF-κB.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

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[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

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2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The polypeptide which has the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5.
[Claim 2] The polypeptide which has the activity which one or more amino acid consists [activity] of deletion and amino acid sequences permuted and/or added in the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and raises the activity of NF-kappa B.
[Claim 3] The polypeptide which has the activity which raises the activity of NF-kappa B, including the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and the amino acid sequence which has 60% or more of homology.
[Claim 4] DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 5] DNA which has the base sequence expressed with either of the array numbers 6-10.
[Claim 6] DNA which carries out the code of the polypeptide which has the activity which it is [activity] DNA according to claim 4 or 5 and DNA hybridized under stringent conditions, and raises the activity of transcription factor NF-kappa B.
[Claim 7] The recombinant vector which includes DNA of a publication in any 1 term of claims 4-6 at a vector, and is obtained.
[Claim 8] The recombinant vector which includes in a vector RNA which becomes any 1 term of claims 4-6 from DNA of a publication, and a homologous array, and is obtained.
[Claim 9] The recombinant vector according to claim 8 whose RNA is a single strand.
[Claim 10] The transformant which holds a recombinant vector according to claim 7.
[Claim 11] The transformant according to claim 10 whose transformant is a transformant chosen from the group which consists of a microorganism, an animal cell, a plant cell, and an insect cell.
[Claim 12] The transformant according to claim 11 whose microorganism is a microorganism belonging to an Escherichia group.
[Claim 13] The transformant according to claim 11 whose animal cell is an animal cell chosen from a mouse myeloma cell, a rat myeloma cell, a mouse hybridoma cell, a CHO cell, a BHK cell, an African green monkey kidney cell, a Namalwa cell, Namalwa KJM-1 cell, a Homo sapiens embryo kidney cell, and a Homo sapiens leukemic cell.
[Claim 14] The transformant according to claim 11 whose insect cell is an insect cell chosen from the ovarian cell of Spodoptera frugiperda, the ovarian cell of Trichoplusia ni, and the ovarian cell of a silkworm.
[Claim 15] The transformant according to claim 10 whose transformant is a nonhuman transgenic animal or a transgenic plant.
[Claim 16] The manufacture approach of this polypeptide which cultivates a transformant given in any 1 term of claims 10-14 to a culture medium, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into a culture, and is characterized by extracting this polypeptide from this culture.
[Claim 17] The manufacture approach of this polypeptide which breeds the nonhuman transgenic animal which holds a recombinant DNA according to claim 7, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into this animal, and is characterized by extracting this polypeptide from the inside of this animal.
[Claim 18] The manufacturing method according to claim 17 characterized by are recording being among the milk of an animal.
[Claim 19] The manufacturing method of this polypeptide which grows the transgenic plant which holds a recombinant DNA according to claim 7, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into this vegetation, and is characterized by extracting this polypeptide from the inside of this vegetation.
[Claim 20] The manufacturing method of this polypeptide characterized by compounding the polypeptide in which this DNA carries out a code by imprint / translation system in in vitro using DNA given in any 1 term of claims 4-6.
[Claim 21] The antibody which recognizes the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 22] The oligonucleotide or this nucleotide which has the array which consists of five to 60 base by which any 1 term of claims 4-6 was followed in the base sequence of DNA of a publication, and the oligonucleotide which has a complementary array.
[Claim 23] How to detect the manifestation including carrying out hybridization to any 1 term of claims 4-6, using DNA or the oligonucleotide according to claim 22 of a publication as a probe of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 24] How to detect the manifestation including performing polymerase chain reaction using the oligonucleotide according to claim 22 as a primer of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 25] How to detect the variation of DNA which carries out the code of the polypeptide given in any 1 term of claims 1-3 by the hybridization method using DNA or the oligonucleotide according to claim 22 of a publication in any 1 term of claims 4-6.
[Claim 26] How to detect the variation including performing polymerase chain reaction using an oligonucleotide according to claim 22 of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 27] An approach given in any 1 term of claims 23-26 used in order to detect the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, or the disease accompanied by unusual cell proliferation.
[Claim 28] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The approach according to claim 27 the disease accompanied by activation of unusual immunocyte is allergy, atopy, asthma, pollinosis, respiratory tract irritation, or an autoimmune disease, and the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor.
[Claim 29] How to control the imprint of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3 characterized by using DNA or the oligonucleotide according to claim 22 of a publication for any 1 term of claims 4-6, or the translation of mRNA.
[Claim 30] How to acquire the promoterregion and the imprint regulatory region of DNA which

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are characterized by using DNA or the oligonucleotide according to claim 22 of a publication for any 1 term of claims 4-6 and which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 31] Physic which contains the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 32] Physic which contains the recombinant vector of a publication in DNA given in any 1 term of claims 4-6, claim 8, or any 1 term of 9.
[Claim 33] Physic containing an antibody according to claim 21.
[Claim 34] Physic containing an oligonucleotide according to claim 22.
[Claim 35] Physic according to claim 31 characterized by a polypeptide having an immunity activation operation.
[Claim 36] Physic according to claim 35 characterized by guiding antitumor activity and antiviral activity through an immunity activation operation.
[Claim 37] Physic given in any 1 term of claims 32-34 whose physic is the physic for the therapy of the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.
[Claim 38] Physic given in any 1 term of claims 32-34 whose physic is the physic for a diagnosis of the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, or the disease accompanied by unusual cell proliferation.
[Claim 39] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, Physic according to claim 37 or 38 whose disease accompanied by unusual cell proliferation it is pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[Claim 40] It is characterized by using the polypeptide of a publication for any 1 term of claims 1-3. The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.
[Claim 41] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent

diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening procedure according to claim 40 whose disease accompanied by unusual cell proliferation it is pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[Claim 42] Physic which acts on a polypeptide given in any 1 term of claims 1-3 acquired by the screening approach according to claim 40 or 41 specifically.
[Claim 43] It is characterized by using the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3 obtained by the approach according to claim 30. The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.
[Claim 44] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening approach according to claim 43 that it is pollinosis, respiratory tract irritation, or an autoimmune disease, the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor, and the disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[Claim 45] Physic which acts on the promoterregion and the imprint regulatory region of DNA which are obtained by the screening approach according to claim 43 or 44, and which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3 specifically.
[Claim 46] The immunological detecting method of a polypeptide given in any 1 term of claims 1-3 characterized by using an antibody according to claim 21.
[Claim 47] The immunity staining method characterized by detecting the polypeptide of a publication in any 1 term of claims 1-3 using an antibody according to claim 21.
[Claim 48] How to screen the matter which controls or promotes the imprint or translation of DNA which is characterized by using an antibody according to claim 21, and which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 49] The manifestation of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3 is a part or the knock out nonhuman animal controlled completely.
[Claim 50] The activity which the polypeptide of a publication has in any 1 term of claims 1-3 is a part or the knock out nonhuman animal controlled completely.
[Claim 51] The screening approach of a variant polypeptide characterized by using the

polypeptide of a publication for any 1 term of claims 1-3 of having dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 52] The variant polypeptide which is obtained by the screening approach according to claim 51 and which has dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 53] DNA which carries out the code of the variant polypeptide according to claim 52.
[Claim 54] The screening approach of a variant polypeptide characterized by using the polypeptide of a publication for any 1 term of claims 1-3 of having the variation which raises this activation to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 55] The variant polypeptide which is acquired by the screening approach according to claim 54 and to which the NF-kappa B activation ability of the polypeptide of a publication went up in any 1 term of claims 1-3.
[Claim 56] DNA which carries out the code of the variant polypeptide according to claim 55.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] DNA which carries out the code of a polypeptide with new this invention, and this polypeptide, The transformant which holds the recombinant DNA which includes this DNA in a vector and is obtained, and this recombinant DNA, The manufacturing method of this polypeptide using this transformant, the analysis method of the amount of manifestations of this DNA and variation which used the oligonucleotide obtained from this DNA, The immunity staining method using the antibody and this antibody which recognize this polypeptide, the activity rise alteration object which introduced variation into this polypeptide by deletion, insertion, a permutation, etc., The dominant negative variant which introduced variation into this polypeptide by deletion, insertion, a permutation, etc., The screening procedure of a compound which fluctuates the activity of this polypeptide, the screening procedure of a compound which fluctuates the manifestation of this DNA, It is related with the compound obtained by the screening procedures of a compound which fluctuate the effectiveness of the imprint by the promotor DNA who manages the imprint of this DNA, and this promotor DNA, and these screening procedures, the knock out animal to which this DNA was suffered a loss or mutated.
[0002]
[Description of the Prior Art] nuclear factor-kappaB (following, NF-kappaB) was identified as a transcription factor to be combined with the enhancer in connection with the immunoglobulin light chain (Ig light chain) gene expression in a B cell in 1986 [Cell, 46, 705-716 (1986), Cell, and 47,921-928 (1986)].
[0003] NF-kappa B consists of heterodimers of two or more molecules belonging to a Rel family, and NF-kappa B generally guided in many cells is considered to be the heterodimer of p50 and RelA [Mol.Cell.Biol., 12, and 674-684 (1992)]. Existence of the factor IkappaB which controls NF-kappa B has also become clear. IkappaB By forming NF-kappa B and complex at the time of no stimulating, and carrying out the mask of the nuclear shift signal of NF-kappa B [Science which has controlled nuclear shift, 242, and 540-546 (1988), Cell, 65, 1281-1289 (1991), Cell, 68, and 1109-1120 (1992), EMBO J., 12, 3893-3901 (1993), Cell, 78, 773-785 (1994), Cell, 87, and 13-20 (1996) --] . the signal transfer molecule which IkappaB will mention later if a cell is stimulated by a tumor necrosis factor alpha (following, TNF-alpha) etc. -- 32 and the 36th serine -- phosphorylation -- it continues, and it is ubiquitin-ized and is decomposed by proteasome. If IkappaB is decomposed, the shift to a nucleus of NF-kappa B will be attained, and it will come to guide various gene expression with an enhancer [Cell, 80, 529-532 (1995), Cell, 80, and 57 3-582 (1995)].
[0004] As the matter which activates NF-kappa B, or a stimulus, cytokine [TNF-alpha, A tumor necrosis factor beta (following, TNF-beta), interleukin 1 alpha (Following and IL-1alpha), interleukin 1 beta (following and IL-1beta)], such as interleukin 2 (the following, IL-2) and a leukemia inhibitor (following, LIF), T cell mitogen (an antigen stimulus, lectin, and an anti-T cell receptor antibody --) Anti-CD2 antibody, anti-CD3 antibody, anti-CD28 antibody, calcium ionophore, B cell mitogen (an anti-IgM antibody, anti-CD40), leukotriene, Lipopolysaccharide (following, LPS), phorbol myristate acetate (Following, PMA), parasitism somesthesis stain, and

virus infection [human immunodeficiency virus (The following, HIV-1), a human T cell leukemia virus 1 (the following, HTLV-1), A hepatitis B virus (following, HBV), an Epatein-Barr virus (The following, EBV), a cytomegalovirus (following, CMV), a herpes simplex virus 1 (The following, HSV-1), a human herpesvirus 6 (the following, HHV-6),], such as Newcastle disease virus (following, NDV), Sendai Virus, and adenovirus, A virus product (double stranded RNA, Tax and HBX, EBNA-2, LMP-1 grade), DNA destructive matter and protein synthesis inhibitor (for example, cycloheximide) Ultraviolet rays, a radiation, oxidation stress, etc. are known [Biochemica et Biophysica Acta, 1072, 63-80 (1991), Annu.Rev.Cell Biol.10, and 405-455 (1994)].

[0005] moreover, as a molecule in which an induction manifestation is carried out by activation of NF-kappa B (1) To an inflammatory response and an immune response at control of a **** molecule group and (2) apotosis **** molecule group, (3) The **** molecule group, the molecule group about (4) viruses, etc. are known by generating and differentiation. [Biochemica et Biophysica Acta, 1072, and 63-80 (199 1), Annu.Rev.Cell Biol.10, 405-455 (1994)], and an induction manifestation are various.
[0006] As a molecule by which an induction manifestation is carried out, specifically Cytokine [IL-1alpha, IL-1beta, IL-2, interleukin 3 (the following, IL-3), interleukin 6 (The following, IL-6), interleukin 8 (the following, IL-8), interleukin 12 (The following, IL-12), TNF-alpha, TNF-beta, interferon beta], a cell growth factor [macrophage colony-stimulating factor (The following, IFN-beta) (The following, M-CSF), a granulocyte macrophage colony-stimulating factor (Following and GM-CSF), granulocyte colony-stimulating factor (following, G-CSF)], A receptor [interleukin 1 receptor (following and IL-1R) antagonist, The interleukin 2 receptor alpha (following and IL-2Ralpha), an immunoglobulin kappa light chain (The following, Ig-kappa-LC), T-cell receptorbeta, a major histocompatibility antigen Classes I and II, beta 2-microglobulin], adhesion factor [endothelialleucocyte adhesionmolecule-1 (The following, MHC) (The following, ELAM-1), vascula r cell adhesionmolecule -1 (Following and VCAM-1) intercellularadhesion molecule-1 (The following, ICAM-1)] and acute stage protein (blood serum amyloid A precursive protein --) Angiotensinogen, the complement factor B, the complement factor C3, the complement factor C4, An induction type NO synthase (following, iNOS), cyclooxygenase 2 (The following, COX-2), a vascular endothelial cell growth factor acceptor (following, VEGF-R2), Transcription factor [c-Rel, p105, I kappa-alpha, c-Myc, an interferon regulator], vimentin, virus [HIV-1, HIV-2, a rhesus monkey immunodeficiency disease virus (The following, IRF-1) (The following, SIVmac), CMV, HSV-1, the rhesus monkey virus 40 (following, SV40), adenovirus], etc. are known [a protein nucleic-acid enzyme, 41, and 1198-1209 (1996)].
[0007] As for the signal transfer about NF-kappa B activation, the elucidation is progressing about TNF-alpha and IL-1. In the activation signal from TNF-alpha A TNF receptor (TNFR1 or TNFR2), TNF receptor-associate d death domain protein (The following, TRADD), TNFR-associated factor -2 (The following, TRAF2), receptor interacting protein (The following, RIP), NF-kappa B-inducing kinase (The following, NIK), IkappaB kinase (following, IKK) [IKKalpha, IKKbeta, IKKgamma (NEMO)], IKK-co mplex-associated protein (following, IKAP), etc. are found out as an activation molecule. [EMBO J., 14, and 2876-288 3 (1995), Science, 267, and 1485-1489 (1995), GENES & DEVELOPMENT, 9, 1586-1597 (1995), Cell, 84, 853-862 (1996), Nature, 388, and 548-554 (1997), Cell, 90,373-383 (1997), Science, 278, and 860-866 (1 997), Science, 278, 866-869 (1997), Cell, 91, 243-252 (1997), Nature, 395, and 292-296 (1998) --] .
[0008] In the activation signal from IL-1 IL-1 recptor 1 (Following and IL-1RI) IL-1 receptor accessory protein (Following and IL-1RAcP), Myd88, IL-1 receptor-associated kinase TNF receptor-associated factor 6 (The following, IRAK) (The following, TRAF6), and TAK1 binding protein 1 [Science by which (the following, TAB1), Transforming gro wth factor-beta-activated kinase 1 (TAK1), etc. are found out as an activation molecule, 270, and 2008-2011 (1995), Nature, 398, 252-256 (1999)].
[0009] It has been thought that the enzyme (NF-kappa B kinase) which phosphorizes NF-kappa B is concerned with enhancement of a NF-kappa B signal on the other hand [J.Biol.Chem.268, 26790-26795 (1993), EMBO J.13, and 4597-4607 (1994)]. As mentioned above, although it is known that very many molecules are participating in activation of NF-kappa B, all the role of the

identified molecules is not solved. In the stimulus of those other than TNF-alpha, such as ultraviolet rays and oxidation stress, or IL-1, the actual condition is that most molecules in connection with activation of NF-kappa B are not solved. furthermore -- even if it sees the tissue specific expression of a Rel family molecule -- an organization -- [Science, 284, 313-316 (1999), Science, 284, 316-320 (1999), Science, 284, 321-325 (1999), Immunity, 10, 421-429 (1999), Nature Genet, 22, and 74-77] the activation device of specific NF-kappa B is expected to be (1999).

[0010] As mentioned above, it is very useful it to be thought for that many [still] strange molecules in the living body concerned with activation of NF-kappa B exist, and to discover and use these genes for the therapy of the disease in which an elucidation or NF-kappa B of symptoms participates. NF-kappa B is bearing the very important role in rise of an immune response in the living body so that the molecule group which carries out an induction manifestation by activation of the molecule group which activates NF-kappa B mentioned above, or NF-kappa B may also show. The cytokine of TNF-alpha which has antitumor or antiviral activity, or IL-1 grade demonstrates a part for the principal part of the operation through activation of NF-kappa B. Moreover, the cytokine which carries out an induction manifestation by NF-kappa B, such as IL-1, IL-2, IL-12, TNF-alpha, and IFN-beta, also rises the immunoreaction in a living body or an organization, and has antitumor or antiviral activity.

[0011] Thus, it is a well-known fact that activation of NF-kappa B controls a neoplasm and a virus in an actual disease, and it is thought that the thing of in the living body or a living body for which the activity of NF-kappa B is artificially raised in an organization in part is very effective in rise of an immune response or enhancement of antitumor and antiviral activity. Therefore, discovery and acquisition of a NF-kappa B activation rise variant are still very more useful in discovery of DNA which carries out the code of the polypeptide and it which activate NF-kappa B and acquisition, and the physic that used antitumor and antivirotic one as the target.

[0012] On the other hand, cytokine, such as IL-1 which carries out an induction manifestation by NF-kappa B, IL-6, IL-8, and TNF-alpha, is also called inflammatory cytokine, and the immune response which rose too much by these cytokine causes various diseases. These cytokine activates a macrophage, neutrophil leucocyte, a lymphocyte, etc., and works towards exacerbation in an inflammatory tissue. Moreover, the adhesion molecules of ELAM-1, VCAM-1, and ICAM-1 grade guided by NF-kappa B [Mol.Cell.Biol. which promotes infiltration in the organization of a leucocyte and rises accumulation of the leucocyte in an inflammatory tissue, 14, and 5701 (1994), Mol.Cell.Biol., 14, 5820 (1994), Pro.Natl.Acad.Sci USA, 90, and 3943 (1993) --] . The enzyme of iNOS or COX-2 grade produces a nitrogen monoxide (following, NO) and prostaglandin E2, respectively, and acts on the escape of acute inflammation or a blood vessel.

[0013] That is, it is thought that NF-kappa B is bearing the central role in acute inflammation and the chronic inflammation through these cells or molecules. Activation of NF-kappa B is actually reported by the synovial membrane of rheumatoid arthritis, the intestinal tract of Crohn's disease, and asthmatic lung tissue. Therefore, in the disease at large in which inflammation, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, chronic hepatitis B, chronic hepatitis C, graft versus host disease, an insulin dependency and non-dependency diabetes mellitus, traumatic brain injury, inflammatory bowel disease, septicemia, and microorganism infection, participates, NF-kappa B is the important target of a symptoms elucidation and remedy development.

[0014] In connection with cancer, EBV is considered for a Burkitt lymphoma (Burkitt lymphoma), the Hodgkin (Hodgkin) disease, T and B, a spontaneous killer cell lymphoma, EBV related gastric cancer, etc. as a cause. TRADD, TRAF, and association are possible for latent membrane protein (the following, LMP1) in which especially EBV carries out a code, a host's NF-kappa B is activated, and it is thought that it is participating in immortalization [EMBO J., 16, 6478-6485 (1997), J.Virology, 69, 2168-2174 (1995), Oncogene, 18, 7161-7167 (1999), Gene Th erapy, and 5,905-912 (1998)]. Moreover, adult T-cell leukemia (adult T-cell leukemia: ATL) Tax infection by HTLV-1 is the cause and especially HTLV-1 carries out [Tax] a code NF-kappa B is activated through association to IkappaB, or activation of IKK. It is thought that apotosis is checked [J.Biol.Chem., 273, 15891-15894 (1999), J.Biol.Chem., 274, and 34417-34424 (1999)]. The various

adhesion molecules which NF-kappa B guides are participating in transition of a cancer cell, and the vascularization through the apotosis inhibition activity and VEGF-R2 by NF-kappa B is participating in growth of a cancer cell. As mentioned above, NF-kappa B is an important innovative drug development or a therapy target also in the field of cancer.

[0015] Furthermore, also in the viral disease which contains NF-kappa B other than cancers, such as an acquired immunode-ficiency syndrome, as a transcription factor, NF-kappa B is an important innovative drug development or a therapy target. Moreover, there is a report called a cause and control of the cellular infiltration also according [ischemia re-reflux failures, such as ischemic encephalopathy,] to NF-kappa B activation and apotosis etc. is considered that NF-kappa B has played the important role in the onset of the disease accompanied by unusual differentiation growth of a smooth muscle cell including arteriosclerosis, the restenosis, etc.

[0016] Although it has been shown clearly that it is what the anti-inflammatory activity of a steroid, the anti-inflammatory activity of aspirin, etc. depend on inhibition of NF-kappa B, there are no drugs screened as what checks specifically [Sceience, 270, 283-286 (1995), Sceience, 270, 286-290 (1995), Molecular and Cellular Biology, 15 and 943-953 (1995)] and NF-kappa B in recent years. It also has many troubles that the drugs known as a thing in connection with inhibition of the existing NF-kappa B have that a side effect is strong, and low selectivity and singularity etc., and compound retrieval to which NF-kappa B was targeted for the purpose of development of a powerful and new antiinflammatory drug with few side effects is performed. As mentioned above, the new polypeptide which activates NF-kappa B is useful on industry, and acquisition of DNA which carries out the code of these polypeptides and it has been called for.

[0017]

[Problem(s) to be Solved by the Invention] This invention Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, traumatic brain injury, The disease accompanied by infection and inflammation of inflammatory bowel disease etc., a Burkitt lymphoma, Hodgkin's disease, The disease accompanied by unusual cell proliferations, such as various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as articular rheumatism and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic infla mmatory response syndrome), Remedies, such as adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome), DNA which carries out the code of a useful polypeptide and this polypeptide to retrieval of a prophylactic and a diagnostic drug and development, It is in offering the antibody which recognizes the gene therapy using the antisense DNA/RNA of this DNA, and this DNA, and this polypeptide, the activity rise alteration object of this polypeptide, the dominant negative variants of this polypeptide, and these directions.

[0018]

[Means for Solving the Problem] As a result of inquiring wholeheartedly in order to solve the above-mentioned technical problem, this invention persons succeed in acquiring DNA which carries out the code of the factor to which activation of NF-kappa B including a new amino acid sequence is urged, and this factor, and came to complete this invention. That is, this invention relates to the following (1) - (54).

[0019] (1) The polypeptide which has the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5.

(2) The polypeptide which has the activity which one or more amino acid consists [activity] of deletion and amino acid sequences permuted and/or added in the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array

numbers 1-5, and raises the activity of NF-kappa B.
[0020] (3) The polypeptide which has the activity which raises the activity of NF-kappa B, including the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and the amino acid sequence which has 60% or more of homology.
(4) (1) DNA which carries out the code of the polypeptide of a publication to any 1 term of - (3).
(5) DNA which has the base sequence expressed with either of the array numbers 6-10.
[0021] (6) DNA which carries out the code of the polypeptide which has the activity which it is [activity] DNA given in (4) or (5), and DNA hybridized under stringent conditions, and raises the activity of transcription factor NF-kappa B.
(7) (4) Recombinant vector which includes DNA of a publication in any 1 term of - (6) at a vector, and is obtained.
(8) (4) Recombinant vector which includes in a vector RNA which becomes any 1 term of - (6) from DNA of a publication, and a homologous array, and is obtained.
[0022] (9) The recombinant vector given in (8) given RNA is a single strand.
(10) The transformant which holds a recombinant vector given in (7).
(11) The transformant given in (10) a given transformant is a transformant chosen from the group which consists of a microorganism, an animal cell, a plant cell, and an insect cell.
(12) The transformant given in (11) a given microorganism is a microorganism belonging to an Escherichia group.
[0023] (13) an animal cell -- a mouse - myeloma -- a cell -- a rat - myeloma -- a cell -- a mouse - a hybridoma -- a cell -- CHO -- a cell -- BHK -- a cell -- an African green monkey - - the kidney -- a cell -- Namalwa -- a cell -- Namalwa KJM - one -- a cell -- Homo sapiens - - an embryo -- the kidney -- a cell -- and -- Homo sapiens -- a leukemic cell -- from -- choosing -- having -- an animal cell -- it is -- (-- 11 --) -- a publication -- a transformant .
(14) The transformant given in (11) a given insect cell is an insect cell chosen from the ovarian cell of Spodoptera frugiperda, the ovarian cell of Trichoplusia ni, and the ovarian cell of a silkworm.
[0024] (15) The transformant given in (10) a given transformant is a nonhuman transgenic animal or a transgenic plant.
(16) (10) The manufacture approach of this polypeptide which cultivates a transformant given in any 1 term of - (14) to a culture medium, is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into a culture, and is characterized by extracting this polypeptide from this culture.
[0025] (17) The manufacture approach of this polypeptide which breeds the nonhuman transgenic animal which holds a recombinant DNA given in (7), is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into this animal, and is characterized by extracting this polypeptide from the inside of this animal.
(18) The manufacturing method given in (17) characterized by are recording being among the milk of an animal.
[0026] (19) The manufacturing method of this polypeptide which grows the transgenic plant which holds a recombinant DNA given in (7), is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into this vegetation, and is characterized by extracting this polypeptide from the inside of this vegetation.
(20) (4) Manufacturing method of this polypeptide characterized by compounding the polypeptide in which this DNA carries out a code by imprint / translation system in in vitro using DNA given in any 1 term of - (6).
[0027] (21) (1) Antibody which recognizes the polypeptide of a publication in any 1 term of - (3).
(22) (4) The oligonucleotide or this nucleotide which has the array which consists of 5 by which any 1 term of - (6) was followed in the base sequence of DNA of a publication - 60 base, and oligonucleotide which has a complementary array.
(23) How to detect the manifestation including carrying out hybridization to any 1 term of - (6), using an oligonucleotide DNA of a publication, or given in (4) (22) as a probe of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3).

[0028] (24) How to detect the manifestation including performing polymerase chain reaction using the oligonucleotide given in (22) as a primer of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3).
(25) How to detect the variation of DNA which carries out the code of the polypeptide given in any 1 term of (1) - (3) by the hybridization method using an oligonucleotide DNA of a publication, or given in (4) (22) in any 1 term of - (6).
[0029] (26) How to detect the variation of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3) which includes performing polymerase chain reaction using an oligonucleotide given in (22).
(27) infection -- inflammation -- following -- a disease -- being unusual -- a smooth muscle cell -- differentiation -- growth -- following -- a disease -- being unusual -- fibroblast -- activation -- following -- a disease -- being unusual -- a synovial membrane -- an organization -- activation -- following -- a disease -- the pancreas -- a beta cell -- a failure -- following -- a disease -- being unusual -- an osteoclast -- activation -- following -- a disease -- being unusual -- immunocyte -- activation -- following -- a disease -- or -- being unusual -- cell proliferation -- following -- a disease -- detecting -- a sake -- using -- (-- 23 --) - (-- 26 --) -- some -- one -- a term -- a publication -- an approach .
[0030] (28) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The approach given in (27) the disease accompanied by activation of unusual immunocyte is allergy, atopy, asthma, pollinosis, respiratory tract irritation, or an autoimmune disease, and the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor.
[0031] (29) How to control the imprint of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3) characterized by using an oligonucleotide DNA of a publication, or given in (4) (22) for any 1 term of - (6), or the translation of mRNA.
(30) How to acquire the promoterregion and the imprint regulatory region of DNA which are characterized by using an oligonucleotide DNA of a publication, or given in (4) (22) for any 1 term of - (6) and which carry out the code of the polypeptide of a publication to any 1 term of (1) - (3).
[0032] (31) (1) Physic which contains the polypeptide of a publication in any 1 term of - (3).
(32) (4) Physic which contains the recombinant vector of a publication in any 1 term of DNA given in any 1 term of - (6), (8), or (9).
(33) Physic containing an antibody given in (21).
(34) Physic containing an oligonucleotide given in (22).
[0033] (35) Physic given in (31) characterized by a polypeptide having an immunity activation operation.
(36) Physic given in (35) characterized by guiding antitumor activity and antiviral activity through an immunity activation operation.
(37) The disease accompanied by infection or inflammation in physic, the disease accompanied by differentiation growth of an unusual smooth muscle cell, The disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, The disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, being unusual -- immunocyte -- activation -- following -- a disease -- being unusual -- cell proliferation -- following -- a disease -- or -- a nerve cell -- a failure -- being based -- a disease -- a therapy -- and/or -- prevention -- a

sake -- physic -- it is -- (-- 32 --) - (-- 34 --) -- some -- one -- a term -- a publication -- physic .

[0034] (38) physic -- infection -- inflammation -- following -- a disease -- being unusual -- a smooth muscle cell -- differentiation -- growth -- following -- a disease -- being unusual -- fibroblast -- activation -- following -- a disease -- being unusual -- a synovial membrane -- an organization -- activation -- following -- a disease -- the pancreas -- a cell -- a failure -- following -- a disease -- being unusual -- an osteoclast -- activation -- following -- a disease -- being unusual -- immunocyte -- activation -- following -- a disease -- or -- being unusual -- cell proliferation -- following -- a disease -- a diagnosis -- a sake -- physic -- it is -- (-- 32 --) - -- (-- 34 --) -- some -- one -- a term -- a publication -- physic .

[0035] (39) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, Physic of (37) or (38) publications whose disease accompanied by unusual cell proliferation are pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.

[0036] (40) (1) It is characterized by using the polypeptide of a publication for any 1 term of - (3). The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.

[0037] (41) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening procedure given [are pollinosis, respiratory tract irritation, or an autoimmune disease, and the given disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor] in (40) the given disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.

[0038] (42) Physic which acts on a polypeptide given in any 1 term of (1) - (3) obtained by the screening approach (40) or given in (41) specifically.

(43) It is characterized by using the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of a publication to any 1 term of (1) - (3) obtained by the approach given in (30). The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease

accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.

[0039] (44) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening approach given in (43) that it is pollinosis, respiratory tract irritation, or an autoimmune disease, the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor, and the disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.

[0040] (45) Physic which acts on the promoterregion and the imprint regulatory region of DNA which are obtained by the screening approach (43) or given in (44), and which carry out the code of the polypeptide of a publication to any 1 term of (1) - (3) specifically.

(46) The immunological detecting method of a polypeptide given in any 1 term of (1) - (3) characterized by using an antibody given in (21).

(47) The immunity staining method characterized by detecting the polypeptide of a publication in any 1 term of (1) - (3) using an antibody given in (21).

[0041] (48) How to screen the matter which controls or promotes the imprint or translation of DNA which is characterized by using an antibody given in (21), and which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3).

(49) (1) The manifestation of DNA which carries out the code of the polypeptide of a publication to any 1 term of - (3) is a part or the knock out nonhuman animal controlled completely.

(50) (1) The activity which the polypeptide of a publication has in any 1 term of - (3) is a part or the knock out nonhuman animal controlled completely.

[0042] (51) The screening approach of a variant polypeptide of having dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of (1)(1) characterized by using polypeptide of publication for any 1 term of - (3) - (3).

(52) the variant polypeptide which has dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of acquisition **** and (1) - (3) by the screening approach given in (51).

(53) DNA which carries out the code of the variant polypeptide given in (52).

[0043] (54) The screening approach of a variant polypeptide of having the variation which is characterized by using the polypeptide of a publication for any 1 term of - (3) and which raises this activation to NF-kappa B activation of the polypeptide of a publication in any 1 term of (1) (1) - (3).

(55) The variant polypeptide which is acquired by the screening approach given in (54) and to which the NF-kappa B activation ability of the polypeptide of a publication went up in any 1 term of (1) - (3).

(56) DNA which carries out the code of the variant polypeptide given in (55).

[0044]

[Embodiment of the Invention] In the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with the polypeptide 2. array numbers 1-5 which have the amino acid sequence chosen from the group which consists of an amino acid sequence

expressed with either of 1. array numbers 1-5 as a polypeptide of this invention one or more amino acid Deletion, The amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the polypeptide 3. array numbers 1-5 which has the activity which it consists [activity] of an amino acid sequence permuted and/or added, and raises the activity of NF-kappa B, and the amino acid sequence which has 60% or more of homology are included. And the polypeptide which has the activity which raises the activity of NF-kappa B can be mentioned.

[0045] The polypeptide which has the amino acid sequence to which one or more amino acid was ****(ed), permuted and/or added in the polypeptide which has the above-mentioned amino acid sequence Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989 (It abbreviates to the 2nd edition of molecular cloning hereafter), Current Protocols in Molecular Biology, John Wiley & Sons, 1987-1997 (It abbreviates to current PUROTO call Inn molecular biology hereafter) Nucleic Acids Research, 10, and 6487 (1982), Proc.Natl.Acad.Sci., USA, 79, and 6409 (1982), Gene, 34, 315 (1985), Nucleic Acids Research, 13, 4431 (1985), The site-specific mutation introducing method of a publication is used for Proc.Natl.Acad.Sc i USA, 82, 488 (1985), etc. For example, it can carry out by introducing site-specific mutation into DNA which carries out the code of the polypeptide which has one amino acid sequence of the array numbers 1-5. although the number of deletion and the amino acid permuted and/or added comes out of 1 partly, and there is and especially the number is not limited -- the technique of common knowledge, such as the above-mentioned site-specific mutation introducing method, -- the number of deletion and extent which can be permuted or added -- it is -- for example, 1- dozens of pieces are 1-5 pieces still more preferably 1-10 pieces more preferably 1-20 pieces.

[0046] Moreover, as a polypeptide of this invention, the amino acid sequence of a publication and the amino acid sequence which has 60% or more of homology are included in either of the array numbers 1-5. The homology with an amino acid sequence given in either of the array numbers 1-5 With analysis software, such as BLAST [J.Mol.Biol., 215, and 403 (1990)] and FASTA (Methods in Enzymology, 183, 63-69) It is most preferably [97% or more of] more preferably desirable [70% or more / 80% or more] at least 60% or more, when it calculates using a default (initialization) parameter 95% or more especially preferably 90% or more still more preferably preferably.

[0047] DNA which has the base sequence expressed with either of the DNA3. array numbers 6-10 which are DNA of the DNA2. claim 4 publication which carries out the code of the polypeptide of a publication to any 1 term of 1. claims 1-3 as DNA of this invention, and DNA hybridized under stringent conditions, and carry out the code of the polypeptide which has the activity which raises the activity of transcription factor NF-kappa B can be mentioned.

[0048] Since two or more sorts of gene codes generally exist per amino acid, it is contained in DNA of this invention, if the code of the polypeptide of this invention is carried out even if either of the array numbers 6-10 is DNA which has a different base sequence. With DNA hybridized under stringent conditions For example, DNA of this inventions, such as DNA which has the base sequence expressed with the array numbers 6, 7, 8, 9, or 10, or some of its fragments are used as a probe. DNA obtained by using a colony hybridization method, a plaque hybridization method, or a Southern blotting hybridization method is meant. The filter which fixed DNA of a colony or the plaque origin is specifically used. The SSC solution of 0.1 - 2 double concentration the bottom of the sodium chloride existence of 0.7 - 1.0 mol/l, and after performing hybridization at 65 degrees C (the SSC solution of concentration 1 time) DNA which can be identified by washing a filter under 65-degree-C conditions can be mentioned using a 150 mmol/l sodium chloride and 15 mmol/l sodium-citrate twist. Hybridization is the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, and D NACloning 1. : It can carry out according to the approach indicated by Core Techniques, A Practical Approach, Second Edition, Oxford University, and 1995 grades.

[0049] As DNA which can be hybridized, specifically When it calculates with analysis software, such as BLAST and FASTA, using a default (initialization) parameter The base sequence expressed with the array numbers 6, 7, 8, 9, or 10, and DNA which has at least 60% or more of

homology, DNA which has 98% or more of homology most preferably can be mentioned especially 95% or more preferably 90% or more still more preferably 80% or more 70% or more.

[0050] Hereafter, this invention is explained to a detail.

1. Preparation Homo sapiens mRNA of DNA of this invention may use a commercial thing (for example, product made from Clontech), and may prepare from human tissue as the following. as the approach of preparing all RNA from an organization -- thiocyanic acid guanidine - trifluoroacetic acid caesium method [Methods in Enzymology, 154, and 3] (19 87) acidity thiocyanic acid guanidine phenol chloroform (AGPC) -- law [Analytical Biochemistry, 162, 156 (1987), the experimental medicine, 9, and 1937 (1991)] etc. is mentioned. Moreover, as an approach of preparing mRNA as polyA+RNA from all RNA, the oligo (dT) fixed cellulose column method (the 2nd edition of molecular cloning) etc. is mentioned. Furthermore, FastTrack mRNA Isolation Kit (product made from Invitrogen), Quick Prep mRNA mRNA can be prepared by using kits, such as Purification Kit (product made from Pharmacia).

[0051] A cDNA library is produced from prepared human tissue mRNA. As a cDNA library producing method, the 2nd edition of molecular cloning, Current PUROTO call Inn molecular biology, A Laboratory Manual, 2 nd Ed., the approach indicated by 1989 grades, (Or a commercial kit, for example, SuperScript Plasmid System for cDNA, Synthesis and Plasmid Cloning (product made from Life Technologies)) The approach using ZAP-cDNA Synthesis Kit (product made from STRATAGENE) etc. is mentioned.

[0052] As a cloning vector for producing a cDNA library, if independence reproduction can be carried out in Escherichia coli K-12, a phage vector, a plasmid vector, etc. can use either. Specifically The product made from ZAP Express[STRATAGENE, Strategies, 5, 58 (1992),], and pBluescript II SK -- (+ [Nucleic Acids Research, 17, and 9494 (1989)]) -- Lambda ZAP II (product made from STRATAGENE), lambdagt10, and lambdagt11 [DNA cloni ng, A Practical Approach, 1, and 49 (1985)], lambdaTriplEx (product made from Clontech), lambdaExCell (product made from Pharmacia), pT7T318U (product made from Pharmacia), pcD2[Mol.Cell.Biol., 3, 280 (1983)], pUC18 [Gene, 33, and 103 (1985)], etc. can be mentioned.

[0053] Either can be used if it is a microorganism belonging to Escherichia coli as a host microorganism. Specifically The product made from Escherichia coli XL1-Blue MRF'[STRATAGENE, Strategies, 5, 81 (1992)], and Escherichia coli C600 [Genetics, 39, and 440 (1954)], Esherichia coli Y1088 [Science, 222, and 778 (1983)], Escherichiacoli Y1090 [Science, 222, and 778 (1983)], Escherichia coli NM522 [J.Mol.Biol., 166, and 1 (1983)], Eshe richia coli K802 [J.Mol.Biol., 16, and 118 (1966)], Escherichia coli JM105 [Gene, 38, and 275 (1985)], etc. are used.

[0054] Although this cDNA library may be used for the following analyses as it is, in order to lower the rate of the imperfect length cDNA and to acquire the perfect length cDNA efficiently if possible Oligo-capping method [Gene which Sugano and others developed, 138, and 171 (1994), Gene, 200, 149 (1997), a protein nucleic-acid enzyme, 41, and 603 (1996), The experimental medicine, 11, 2491 (1993), and cDNA cloning, Yodosha (1996) Method of producing a gene library, Yodosha (1994) The cDNA library prepared using] may be used for the following analyses.

[0055] The base sequence of this DNA is determined by isolating each clone from the produced cDNA library, and analyzing the base sequence of cDNA from an end using base sequence analysis apparatus, such as the base sequence analysis approach usually used, for example, the dideoxy chain termination method of Sanger and others (Sanger), [Proc.Natl.Acad.Sci.USA, 74, 54 63 (1977)], and ABI PRISM377 DNA sequencer (product made from PEBiosystem), about each clone. By translating the acquired base sequence into an amino acid sequence, the amino acid sequence of the polypeptide in which this DNA carries out a code can be acquired.

[0056] Moreover, the base sequence from which the acquired base sequence was acquired [whether it is a new base sequence and], and a base sequence with homology can be searched by comparing the acquired base sequence using homology analyzers, such as a base sequence in base sequence databases, such as GenBank and EMBL, BLAST, and FASTA. Moreover, the family protein suddenly presumed also in the polypeptide in which the base sequence carries out a code and a polypeptide with homology, for example, the polypeptide originating in the corresponding gene in living thing kind with an another rat, the same activity, and the same

function can be searched by comparing the amino acid sequence acquired from the base sequence with amino acid sequence databases, such as SwissProt, PIR, and GenPept.
[0057] Based on the base sequence of the homologous gene which became clear by database retrieval, a specific primer is designed in this gene and PCR is performed by using as mold the single strand cDNA acquired as mentioned above or a cDNA library. When a magnification fragment is obtained, subcloning of this fragment is carried out to a suitable plasmid. subcloning -- a magnification fragment -- as it is -- or a restriction enzyme and DNA polymerase -- after processing and a law -- it can carry out by including in a vector by the method. As a vector, pBluescript SK (-), (the product made from Stratagene), pBluescript II SK (+), (the product made from Stratagene), pDIRECT [Nucreic Acid s Research, 18, and 6069 (1990)], pCR-Script Amp SK (+), (the product made from Stratagene), pT7Blue (product made from Novagen), pCRII (product made from Invitrogen), pCR-TRAP (product made from Genehunter), pNo TAT7 (5'->3' company make), etc. can be mentioned.
[0058] After DNA which consists of one base sequence of the array numbers 6-10 is once acquired and the base sequence is determined, DNA of this invention is acquirable by preparing the primer based on the base sequence of 5 'edge and 3' edge of this base sequence, and amplifying DNA using cDNA or the cDNA library compounded from mRNA contained in the tissue or the cell of Homo sapiens or a nonhuman animal.
[0059] Moreover, DNA of this invention is acquirable by performing colony hybridization and plaque hybridization (the 2nd edition of molecular cloning) to cDNA or the cDNA library compounded from mRNA contained in the tissue or the cell of Homo sapiens or a nonhuman animal by using as a probe an overall length or a part of DNA which consists of one base sequence of the array numbers 6-10.
[0060] DNA of this invention is also acquirable by carrying out chemosynthesis based on the base sequence of determined DNA with DNA synthesis machines, such as a DNA synthesis machine (model 392) of Perkin-Elmer using a HOSUFO aminodite method. As an oligonucleotide of this invention, the derivative (henceforth, derivative oligonucleotide) of oligonucleotides, such as Oligo DNA and Oligo RNA, and this oligonucleotide etc. is mentioned.
[0061] as this oligonucleotide or this oligonucleotide, and the oligonucleotide (henceforth, antisense oligonucleotide) equivalent to a complementary array -- for example, in some base sequences of mRNA to detect, the sense primer equivalent to the base sequence by the side of a five prime end, the antisense primer equivalent to the base sequence by the side of a three-dash terminal, etc. can be mentioned. However, the base which is equivalent to a uracil in mRNA serves as thymidine in an oligonucleotide primer.
[0062] As a sense primer and an antisense primer, it is the oligonucleotide which does not change extremely both melting out temperature (Tm) and number of bases, and the thing of the number of 10 - 50 bases is mentioned preferably five to 60 base. What was exchanged for HOSUFORO thioate association in the phosphodiester bond in an oligonucleotide as a derivative oligonucleotide, That from which the phosphodiester bond in an oligonucleotide was changed into N3'-P5' HOSUFO friend date association, That from which RIPOSU and the phosphodiester bond in an oligonucleotide were changed into peptide nucleic-acid association, That by which the uracil in an oligonucleotide was permuted by the C-5 propynyl uracil, That by which the uracil in an oligonucleotide was permuted by the C-5 thiazole uracil, That by which the cytosine in an oligonucleotide was permuted with the C-5 propynyl cytosine, That by which the cytosine in an oligonucleotide was permuted with the phenoxazine qualification cytosine (phenoxazine-modified cytosine), That by which the ribose in an oligonucleotide was permuted by the 2'-methoxyethoxy ribose is mentioned [a cell technology, 16, and 1463 (1997)].
[0063] 2. In host cell this invention used for the detecting method (1) activity detection of NF-kappa B activation of DNA of this invention, if it is the cell which can introduce DNA into intracellular as a host cell used in order to detect the activity of DNA, any cells can be used. As this cell, the cell originating in for example, bacteria and Archea, algae, a fungus, vegetation, an animal, etc. is mentioned. Specifically, the cell of the following living thing origin is mentioned.
[0064] Escherichia coli, Bacillus subtilis, etc. are mentioned as bacteria and Archea. The cyanobacterium of a Synechococcus group or a Synechocystis group etc. is mentioned as algae.

As vegetation, tobacco, Arabidopsis, a tomato, a potato, the rapeseed, cotton, soybeans, a rice, or corn is mentioned. Saccharomyces cerevisiae, Aspergillus nigar, etc. are mentioned as a fungus. Mammalian, Arthropoda, etc. are mentioned as an animal.
[0065] As mammalian, Homo sapiens, an ape, a mouse, a rat, a guinea pig, or a mink is mentioned. Specifically as a human cell, the T cell stock Jurkat [the cell strain of number TIB-512 of an American type culture collection (it is hereafter written as ATCC)], the B cell stock Namalwa (ATCC CRL-1432), the uterine cancer cell strain Hela (ATCC CCL-2), the nephrocyte stock 293 [J.Gen.Viol.36 and 59-72 (1977)], etc. can be used. As a cell of mammalians other than Homo sapiens, ape nephrocyte stock COS-1 (ATCC CRL-16 50), Ape nephrocyte stock COS-7 (ATCC CRL-1651), the Chinese hamster ovary cell (Chinease Hamster Ovary) cell strain CHO (ATCC CRL-9096, ATCC CCL-61), Mouse cell strain Ba/F3 (RIKEN Cell Bank RCB0805), The mouse cell strain L929 (RIKEN Cell Bank RCB0081), rat cell strain NRK-49F (ATCC CRL-1570), the mink cell strain MvlLu (ATCC CCL-64), etc. can be used. A silkworm is mentioned as Arthropoda. Specifically, nine shares of Spodoptera frugiperda Sf, 21 shares of Sf(s), etc. can be used. When retrieval of DNA used as the screening target of the protein nature drugs for a therapy or drugs is the purpose, it is desirable to make the cell of mammalian, especially a human cell into a host.
[0066] (2) If it is the approach of introducing a gene into a host cell as an approach of introducing DNA of transgenics method this invention to a host cell into a host cell, it can use by any approaches. For example, the electroporation method (the Yodosha biotechnology manual series 4 and 23), A calcium phosphate method (the Yodosha biotechnology manual series 4 and 13), The DEAE dextran method (the Yodosha biotechnology manual series 4 and 16), The RIPOFE cushion method (the Yodosha biotechnology manual series 4 and 28), A microinjection method (the Yodosha biotechnology manual series 4 and 36), Well-known approaches, such as the adenovirus method (the Yodosha biotechnology manual series 4 and 43) and the vaccinia virus method (Yodosha biotechnology manual series 4 and 59) retrovirus vector method (the Yodosha biotechnology manual series 4 and 74), can be used.
[0067] (3) Since DNA of approach this invention which acquires DNA of this invention can activate NF-kappa B by making it discovered in a cell, it can acquire DNA of this invention by using the approach of detecting activation of NF-kappa B in a cell. The following approaches are mentioned as an approach of detecting activation of NF-kappa B.
[0068] For example, the approach of analyzing association to imprint regulatory region by the gel shifting method (the Yodosha biotechnology manual series 5 and 107) etc., and the method of detecting the phosphorylation of IkappaB and ubiquitin-ization by western blotting (the Yodosha biotechnology manual series 7 and 179) etc. are mentioned as an approach using a cell extract. Furthermore, the approach of detecting using a reporter gene as an approach of detecting efficiently can be mentioned. As a reporter gene, the gene which carries out the code of luciferase, the Homo sapiens placenta alkaline phosphatase, the beta-galactosidase, urokinase, chloramphenicol acetyltransferase, a human growth hormone, various Greenfluorescent protein (following, GFP), etc. can be used. If it is the promotor who is imprinted by NF-kappa B and gets as a promotor who connects with a reporter gene, any promotors can use. For example, the promotor DNA fragment isolated by starting the promoterregion of a gene where the manifestation is controlled by activation of NF-kappa B by restriction enzyme digestion from Chromosome DNA, the promotor DNA fragment obtained by amplifying by the PCR method by using Chromosome DNA as mold, or the synthetic DNA fragment which has this promotor's base sequence is mentioned.
[0069] Specifically IL-1alpha, IL-1beta, IL-2, IL-3, IL-6, IL-8, IL-12, TNF-alpha, TNF-beta, IFN-beta, M-CSF, GM-CSF, G-CSF, L-2Ralpha, Ig-kappa-LC, T-cell receptorbeta, the MHC class 1, beta 2-microglobulin, LAM-1, VCAM-1, ICAM-1, blood serum amyloid A precursive protein, Angiotensinogen, the complement factor B, the complement factor C3, the complement factor C4, iNOS, COX-2, VEGF-R2, c-Rel, p105, IkappaBalpha, Promotors, such as c-Myc, IRF-1, HIV-1, HIV-2, SIVmac, CMV, HSV-1, SV40, and adenovirus, a synthetic promotor with [one or more] those consensus sequences, etc. are mentioned.
[0070] By the detection approach using a reporter gene, after producing the imprint unit which

connected the reporter gene with the above-mentioned promotor, the cell strain which included the imprint unit in the chromosome of a host cell is produced. After introducing into intracellular [this] the unit which discovers DNA of this invention and making DNA of this invention discover, activation of NF-kappa B is detectable by measuring the amount of manifestations of a reporter gene. Or after producing the imprint unit which connected the reporter gene with the above-mentioned promotor, activation of NF-kappa B is detectable by introducing into coincidence two units, this imprint unit and the unit which discovers DNA of this invention, at a host cell, and measuring the amount of manifestations of a reporter gene.

[0071] 3. Using the approach indicated by the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, etc., by the following approaches, it can be made discovered in a host cell and the polypeptide of manufacture this invention of the polypeptide of this invention can manufacture DNA of this invention.

[0072] The DNA fragment of the suitable die length containing the part which carries out the code of this polypeptide if needed based on an overall length cDNA is prepared. A recombination vector is produced by inserting this DNA fragment or an overall length cDNA in the lower stream of a river of the promotor of a suitable expression vector. The transformant which produces the polypeptide of this invention can be obtained by introducing this recombination vector into the host cell which suited this expression vector.

[0073] As a host cell, if bacteria, yeast, an animal cell, an insect cell, a plant cell, etc. can discover the gene made into the purpose, all can use them. As an expression vector, in the above-mentioned host cell, the nest to the inside of a chromosome is possible, and autonomous replication's being possible or the thing containing a promotor is used for the location which can imprint DNA which carries out the code of the polypeptide of this invention.

[0074] When using procaryotes, such as bacteria, as a host cell, while the recombination vector which comes to contain DNA which carries out the code of the polypeptide of this invention can be replicated autonomously in a procaryote, it is desirable that they are a promotor, a ribosome junction sequence, the gene that carries out the code of the polypeptide of this invention, and the vector which consisted of conclusion arrays of an imprint. In addition, the gene which controls a promotor may be contained in the vector.

[0075] As an expression vector, for example pBTrp2 (product made from Boehrin ger Mannheim), pBTac1 (product made from Boehringer Mannheim), pBTac2 (product made from Boehringer Mannheim), pKK 233-2 (product made from Pharmacia), pSE280 (product made from Invitrogen), pGEMEX-1 (product made from Promega), pQE-8 (product made from QIAGEN), pKYP10 (Provisional-Publication-No. 5 8-110600 No.) and pKYP200 [Agricultural.Biological.Chemistry., 48, and 669 (1984)], pLSA1 [Agric.Bil o.Chem., 53, and 277 (1989)], pGEL1 [Proc.Natl.Acad.Sci.USA, 82, and 4306 (1985)], pBluescript II SK (-), (the product made from Stratagene), From pTrS30[Escherichia coli JM109/pTrS30 (FERM BP-5407), preparation], From pTrS32[Escherichia coli JM109/pTrS32 (FERM BP-5408), preparation], It prepares from pGHA2 [Escherichia coli IGHA2 (FER M BP-400). It prepares from JP,60-221091,A] and pGKA2 [Escherichia coli IGKA2 (FERM BP-6798). JP,60-221091,A] and pTerm2 (U.S. Pat. No. 4,686,191 --) U.S. Pat. No. 4,939,094 and U.S. Pat. No. 5,160,735, pSupex, and pUB110, pTP5, pC194 and pEG400 [J.Bacteriol., 172, and 2392 (1990)], As a . expression vector which can mention pGEX (product made from Pharmacia), a pET system (product made from Novagen), etc. It is desirable to use what adjusted between the Shine-Dalgarno (Shine-Dalgarno) arrays and initiation codons which are a ribosome junction sequence in a suitable distance (for example, six to 18 base).

[0076] As a promotor, as long as it can be discovered in a host cell, what kind of thing may be used. For example, the promotor originating in Escherichia coli, phage, etc., such as a trp promotor (Ptrp), a lac promotor, PL promotor, PR promotor, and T7 promotor, and SPO1 promotor, SPO2 promotor, a penP promotor, etc. can be mentioned. Moreover, the promotor by whom the design alteration was artificially done like the promotor (Ptrpx2) who did 2 serials of the Ptrp, a tac promotor, lacT7 promotor, and a letI promotor [Gene, 44, and 29 (1986)] can use.

[0077] The production rate of the polypeptide made into the purpose can be raised by permuting a base so that it may become the optimal codon for a host's manifestation about the base sequence of the part which carries out the code of the polypeptide of this invention. In the

recombination vector of this invention, although the conclusion array of an imprint is not necessarily required for the manifestation of DNA of this invention, it is desirable to arrange the conclusion array of an imprint directly under a structural gene.

[0078] As a host cell, Escherichia, Serratia, Bacillus, Brevibacterium, The microorganism belonging to Corynebacterium, Microbacterium, Pseudomonas, etc., For example, Escheri chia coli XL1-Blue, Escherichia coli XL2-Blue, Escherichia coli DH1, Escherichia coli MC1000, Escherichia coli KY3276, Escherichia coli W1485, and Escherichia coli JM109, Escherichia coliHB101, Escherichia coliNo.49, Escherichia coli W3110 and Escherichia coliNY49, Serr atia ficaria, Serratia fonticola, Serratialiquefaciens, Serratia marcescens, Baci llus subtilis, Bacillus amyloliquefacines, Brevibacterium ammoniagenes, Brevibacterium immariophilum ATCC14068 and Brevibacterium saccharolyticum ATCC14066, Brevibacterium flavum ATCC14067, Brevibacterium lactofermentum ATCC13869, and Corynebacterium glutamicum ATCC1303 2, Microbacterium ammoniaphilum ATCC15354, and Pseudomonasu sp.D-0110 grade can be mentioned.

[0079] All can be used if it is the approach of introducing DNA to the above-mentioned host cell as the introductory approach of a recombination vector. For example, the approach using calcium ion [Proc.Natl. Acad.Sci.USA, 69, and 2110 (1972)], The approach of a publication etc. can be mentioned to the protoplast method (JP,63-248394,A) or Gene, 17, 107 (1982) and Mole cular & General Genetics, 168, and 111 (1979).

[0080] When using yeast as a host cell, YEP13 (ATCC37115), YEp24 (ATCC37051), YCp50 (ATCC37419), pHS19, and pHS15 grade can be mentioned as an expression vector. As a promotor, as long as it can be discovered in a yeast-fungus stock, which thing may be used, for example, they are the promotor of the gene of glycolytic pathways, such as a hexose kinase, PHO5 promotor, a PGK promotor, a GAP promotor, an ADH promotor, gal1 promotor, gal10 promotor, a heat shock protein promotor, and MF1. A promotor, CUP1 promotor, etc. can be mentioned.

[0081] As a host cell, the microorganism belonging to a Saccharomyces, a clew IBERO married-woman group, the Trichosporon, a SHUWANIO married-woman group, etc., for example, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyce s lactis, Trichosporon pullulans, Schwanniomyces alluvius, etc. can be mentioned. All can be used if it is the approach of introducing DNA into yeast as the introductory approach of a recombination vector. For example, the electroporation method [Methods.Enzymol., 194, and 182 (1990)], The spheroplast method [Proc.Natl.Acad.Sci.USA, 84, and 1929 (1978)], The acetic-acid lithium method [J.Bacteriolog y, 153, and 163 (1983)], an approach given in [Proc.Natl.Acad.Sci.USA, 75, and 1929 (1978)], etc. can be mentioned.

[0082] In using an animal cell as a host, as an expression vector For example, pcDNAI, pcDM8 (Funakoshi Co., Ltd. make), pAGE107 [JP,3-22979,A;Cytotechnology, 3, and 1 33 (1990)], pAS 3-3 (JP,2-227075,A) and pCDM8 [Nature, 329, and 840 (1987)], pcDNAI/A mp (product made from Invitrogen), pREP4 (product made from Invitrogen) and pAGE103 [J.Biochemistry, 101, and 1307 (1987)], and pAGE210 grade can be mentioned.

[0083] As a promotor, if it can be discovered in an animal cell, all can be used, for example, the promotor of IE (immediate early) gene of a cytomegalovirus (CMV), the initial promotor of SV40, the promotor of a retrovirus, a metallothionein promotor, a heat shock promotor, SRalpha promotor, etc. can be mentioned. Moreover, the enhancer of Homo sapiens's CMV IE gene may be used with a promotor.

[0084] As a host cell, the NAMARUBA (Namalwa) cell which is a human cell, the COS cell which is a cell of an ape, the CHO cell which is a cell of a Chinese hamster, HBT5637 (JP,63-299,A), etc. can be mentioned. If it is the approach of introducing DNA into an animal cell as the introductory approach of a recombination vector, all can be used, for example, the electroporation method [Cytotechnology, 3, and 133 (1990)], a calcium phosphate method (JP,2-227075,A), the RIPOFE cushion method [Proc.Natl.Acad.Sci.USA, 84, and 7413 (1987)], etc. can be mentioned.

[0085] When using an insect cell as a host, the polypeptide of this invention can be discovered by the approach indicated by the current PUROTO call Inn molecular biology supplement 1-38 (1

987-1997), Baculovirus Expression Vectors, A Laboratory Manual, W.H.Freeman and Company, NewYork (1992), Bio/Technology, 6, 47, etc. (1988).
[0086] That is, after carrying out cointroduction of a recombination gene installation vector and the baculovirus to an insect cell, rearranging in insect cell culture supernatant liquid and obtaining a virus, it can rearrange further, a virus can be infected with an insect cell, and the polypeptide of this invention can be made to discover. As a transgenics vector used in this approach, pVL1392, pVL1393, pBlueBacIII (both product made from Invitorogen), etc. can be mentioned, for example.
[0087] As a baculovirus, the out GURAFA KARIFORUNIKA NUKUREA poly sludge cis- virus (Autographa californica nuclear polyhedrosis virus) which is a virus infected with the department insect of a cutworm can be used, for example. As an insect cell, Sf9 and Sf21 which are the ovarian cell of Spodoptera frugiperda [Baculovirus Expression Vectors, A Laboratory Manual, W.H.Freeman and Company, and New York] (1992), High5 (product made from Invitrogen) which is the ovarian cell of Trichoplusia ni can be used.
[0088] As the cointroduction approach of of the above-mentioned recombination gene installation vector to an insect cell and the above-mentioned baculovirus for preparing a recombination virus, a calcium phosphate method (JP,2-2270,A 75), the RIPOFE cushion method [Proc.Natl.Acad.Sci.USA, 84, 7413 (1987)], etc. can be mentioned, for example. When using a plant cell as a host cell, a Ti plasmid, a tobacco mosaic virus vector, etc. can be mentioned as an expression vector.
[0089] As a promotor, if it can be discovered in a plant cell, which thing may be used, for example, 35S promotor of a cauliflower mosaic virus (CaMV), rice actin 1 promotor, etc. can be mentioned. As a host cell, plant cells, such as tobacco, a potato, a tomato, a ginseng, soybeans, rape, alfalfa, a rice, wheat, and a barley, etc. can be mentioned.
[0090] If it is the approach of introducing DNA into a plant cell as the introductory approach of a recombination vector, all can be used, for example, Agrobacterium (Agrobacterium) (JP,59-140885,A, JP,60-70080,A, WO 94/00977), the electroporation method (JP,60-251887,A), the approach (the 2606856th patent 2517813rd of a patent) using party Kurgan (gene gun), etc. can be mentioned.
[0091] As the gene expression approach, secretory production, a fusion polypeptide manifestation, etc. can be performed according to the approach indicated by the 2nd edition of molecular cloning in addition to a direct manifestation. When it is made discovered by yeast, the animal cell, the insect cell, or the plant cell, the polypeptide to which sugar or a sugar chain was added can be obtained.
[0092] This polypeptide can be manufactured by cultivating the transformant incorporating DNA of this invention which rearranges and holds an expression vector to a culture medium, carrying out generation are recording of the polypeptide of this invention into a culture, and extracting this polypeptide from this culture. As a culture medium which cultivates the transformant obtained considering eukaryotes, such as procaryotes, such as Escherichia coli, or yeast, as a host, the carbon source in which this living thing can carry out utilization, a nitrogen source, mineral, etc. are contained, and as long as it is the culture medium which can cultivate a transformant efficiently, any of a natural medium and a synthetic medium may be used.
[0093] Alcohols, such as organic acids, such as carbohydrates, such as a glucose, fructose, a sucrose, molasses containing these, starch, or starch hydrolysate, an acetic acid, and a propionic acid, ethanol, and propanol, etc. can be used that what is necessary is just that in which this living thing can carry out utilization as a carbon source. As a nitrogen source, the ammonium salt of inorganic acids, such as ammonia, an ammonium chloride, an ammonium sulfate, ammonium acetate, and ammonium phosphate, or an organic acid, other nitrogen-containing compounds and a peptone, a meat extract, a yeast extract, corn steep liquor, casein hydrolysate, soybean cake and soybean cake hydrolyzate, various fermentation fungus bodies, the digest of those, etc. can be used.
[0094] As mineral salt, the first potassium of a phosphoric acid, the second potassium of a phosphoric acid, magnesium phosphate, magnesium sulfate, a sodium chloride, a ferrous sulfate, a manganese sulfate, a copper sulfate, a calcium carbonate, etc. can be used. Culture is usually

performed under aerobic conditions, such as shaking culture or deep part aeration spinner culture. Culture temperature has good 15-40 degrees C, and culture time amount is usually for 16 hours - seven days. pH under culture is held to 3.0-9.0. Adjustment of pH is performed using an inorganic or organic acid, an alkali solution, a urea, a calcium carbonate, ammonia, etc.
[0095] Moreover, antibiotics, such as ampicillin and a tetracycline, may be added to a culture medium if needed during culture. When cultivating as a promotor the microorganism using an inductive promotor which was rearranged and carried out the transformation by the vector, an inducer may be added to a culture medium if needed. For example, when cultivating the microorganism which used the trp promotor for isopropyl-beta-D-thio galactopyranoside (IPTG) etc. when cultivating the microorganism using a lac promotor which was rearranged and carried out the transformation by the vector and which was rearranged and carried out the transformation by the vector, the Indore acrylic acid (IAA) etc. may be added to a culture medium.
[0096] As a culture medium which cultivates the transformant obtained considering the animal cell as a host RPMI1640 culture medium currently generally used [The Journal of the American Medical Association, 199, and 519 (1967)], The MEM culture medium of Eagle [Science, 122, and 501 (1952)], A Dulbecco alteration MEM culture medium [Virology, 8, and 396 (1959)], The culture medium which added fetal calf serum etc. can be used for 199 culture media [Proceeding of the Society for the Biolog ical Medicine, 73, and 1 (1950)] or these culture media. culture -- usually -- pH 6-8, 30-40 degrees C, and 5%CO -- it carries out for one - seven days under lower conditions 2 ****. Moreover, antibiotics, such as a kanamycin and penicillin, may be added to a culture medium if needed during culture.
[0097] As a culture medium which cultivates the transformant obtained considering the insect cell as a host, the TNM-FH culture medium (product made from Pharmingen) currently generally used, a Sf-900 II SFM culture medium (product made from Life Technologies), ExCell400 and ExCell405 (all are the products made from JRH Biosciences), Grace's Insect Medium [Nature, 195, and 788 (1962)], etc. can be used. Culture is usually performed for one - five days under conditions, such as pH 6-7 and 25-30 etc. degrees C. Moreover, antibiotics, such as gentamycin, may be added to a culture medium if needed during culture.
[0098] A plant cell can be made to be able to specialize in the cell and organ of the vegetation as a cell, and the transformant obtained as a host can cultivate it. As a culture medium which cultivates this transformant, auxin, cytokinin, etc. can use the culture medium which added plant hormone for Murashige - currently generally used and - SUKUGU (MS) culture medium, the White (White) culture medium, or these culture media. Culture is usually performed for three - 60 days under pH 5-9 and 20-40-degree C conditions. Moreover, antibiotics, such as a kanamycin and hygromycin, may be added to a culture medium if needed during culture.
[0099] This approach can be chosen by there being an approach which it makes host intracellular produce, an approach of making it secrete out of a host cell, or the approach of making it produce on a host cell envelope as a process of the polypeptide of this invention, and changing the host cell to be used and the structure of a polypeptide made to produce. When the polypeptide of this invention is produced on host intracellular or a host cell envelope, Paulson's and others approach [J.Biol.Chem., 264, and 17619 (1989)], Approach [Proc.Natl.Acad.Sci.USA of a low and others, 86, and 8227 (1989), This polypeptide can be made to secrete positively out of a host cell by applying the approach of a publication correspondingly to Genes Develop., 4, 1288 (1990)] or JP,5-336963,A, and WO94 / 23021 grades.
[0100] That is, the polypeptide of this invention can be made to secrete positively out of a host cell by making it discovered in the form which added transit peptide before the polypeptide including the active site of the polypeptide of this invention using the transgenic technique. Moreover, according to the approach indicated by JP,2-227075,A, a volume can also be raised using the gene amplification system using a dihydrofolate reductase gene etc.
[0101] Furthermore, by making the cell of the animal which carried out transgenics, or vegetation redifferentiate, the animal individual (transgenic nonhuman animal) or vegetable individual (transgenic plant) into which the gene was introduced can be developed, and the polypeptide of this invention can also be manufactured using these individuals. When a transformant is an

animal individual or a vegetable individual, this polypeptide can be manufactured by breeding or growing, carrying out generation are recording of this polypeptide according to the usual approach, and extracting this polypeptide from this animal individual or a vegetable individual.
[0102] The method of producing the polypeptide of this invention is mentioned into the animal which introduced and developed the gene as an approach of manufacturing the polypeptide of this invention using an animal individual, for example according to the well-known approach [American Journal of Clinical Nutrition, 63, 639S (1996), American Journal of Clinical Nutrition, 63, 627S (1996), Bio/Technology, 9, and 830 (1991)].
[0103] In the case of an animal individual, this polypeptide can be manufactured by breeding the transgenic nonhuman animal which introduced DNA which carries out the code of the polypeptide of this invention, generating and storing up this polypeptide into this animal, and extracting this polypeptide from the inside of this animal. As an are recording location in this animal, the milk (JP,63-309192,A) of this animal, an egg, etc. can be mentioned, for example. under the present circumstances -- although all can be used as a promotor boiled and used if it can be discovered for an animal -- an alveolar epithelial cell -- specific alpha casein promotor who is a promotor, beta casein promotor, a beta lactoglobulin promotor, a whey acidity protein promotor, etc. are used suitably.
[0104] As an approach of manufacturing the polypeptide of this invention using a vegetable individual For example, well-known approach [tissue culture and 20 (1994), the transgenic plant which introduced DNA which carries out the code of the polypeptide of this invention It grows according to tissue culture, 21 (1995), Trends in Biotechnology, 15, and 45 (1997)]. The method of producing this polypeptide is mentioned by generating and storing up this polypeptide into this vegetation, and extracting this polypeptide from the inside of this vegetation.
[0105] When the polypeptide of this invention is discovered in the state of the dissolution to intracellular, the polypeptide manufactured by the transformant of this invention collects cells according to centrifugal separation after culture termination, crushes a cell by the ultrasonic crusher, the French press, the MANTONGAURIN homogenizer, dynomill, etc. after suspending in the drainage system buffer solution, and obtains a cell-free extract. The isolation purification method of an enzyme usual from the supernatant liquid obtained by carrying out centrifugal separation of this cell-free extract, Namely, the salting-out method by the solvent extraction method, an ammonium sulfate, etc., the desalting method, settling by the organic solvent, The anion-exchange chromatography method using resin, such as diethylaminoethyl (DEAE)-sepharose and DIAIONHPA-75 (Mitsubishi Kasei Corp. make), The cation-exchange chromatography method using resin, such as S-Sepharose FF (product made from Pharmacia), The hydrophobic chromatography method using resin, such as butyl sepharose and phenyl sepharose, independent in technique, such as electrophoresis methods, such as gel filtration using molecular sieving, the affinity chromatography method, the chromatofocusing method, and isoelectric focusing, -- or it can combine and use and a purification preparation can be obtained.

[0106] Moreover, when this polypeptide forms an insoluble object in intracellular and is discovered, the insoluble objects of a polypeptide are collected as a precipitate fraction by crushing after collecting cells similarly and performing centrifugal separation. The collected insoluble object of a polypeptide is solubilized with a protein modifier. After returning this polypeptide to a normal spacial configuration by diluting or dialyzing this solubilization liquid, the purification preparation of this polypeptide can be obtained according to the same isolation purification method as the above.
[0107] When derivatives, such as a polypeptide of this invention or its sugar qualification object, are secreted out of a cell, derivatives, such as this polypeptide or its sugar chain adduct, can be collected to a culture supernatant. That is, a purification preparation can be obtained by acquiring a soluble fraction and using the same isolation purification method as the above from this soluble fraction by processing this culture by technique, such as the same centrifugal separation as the above.
[0108] moreover, the polypeptide of this invention -- Fmoc -- law (fluorenyl methyloxy carbonyl process) and tBoc -- it can manufacture also by chemosynthesis methods, such as law (t-

butyloxy carbonyl process). Moreover, chemosynthesis can also be carried out using peptide synthesis machines, such as Advanced ChemT ech, Perkin-Elmer, Amersham Pharmacia Biotech, Protein Tec hnology Instrument, Synthecell-Vega, PerSeptive, and Shimadzu.
[0109] 4. Antibodies which recognize the polypeptide of this invention, such as a polyclonal antibody and a monoclonal antibody, are producible by using as an antigen the synthetic peptide which has some amino acid sequences of the purification preparation of the partial fragment polypeptide of the polypeptide of preparation this invention of the antibody which recognizes the polypeptide of this invention, or this polypeptide, or the polypeptide of this invention.
[0110] (1) A polyclonal antibody is producible by medicating the inside of hypodermically [of an animal], and a vein, or intraperitoneal with a suitable adjuvant (for example, [Freund's complete adjuvant (Complete Freund's Adjuvant) or aluminium hydroxide gel, a pertussis vaccine], etc.), using as an antigen the peptide which has some amino acid sequences of the overall length of the polypeptide of production this invention of a polyclonal antibody, the purification preparation of the partial fragment polypeptide of this polypeptide, or the polypeptide of this invention.
[0111] As an animal prescribed for the patient, a rabbit, a goat, the rat of three to 20 weeks old, a mouse, a hamster, etc. can be used. The dose of this antigen has desirable 50-100microper animal g. When using a peptide, it is desirable to use as an antigen what carried out covalent bond of the peptide to carriea protein, such as a SUKASHI guy hemocyanin (keyhole limpet haemocyanin) and cow thyroglobulin. The peptide used as an antigen is compoundable with a peptide synthesis machine.
[0112] Administration of this antigen is performed 3 to 10 times every one - two weeks after the 1st administration. It will collect blood from an eyegrounds venous plexus after each administration on three - the 7th, and will check that this blood serum reacts with the antigen used for immunity with enzyme immunoassay [enzyme immunoassay (ELISA method):********** (1976), Antibodies-A Laboratory Manual, and Cold Spring Harbor Laboratory (1988)] etc.
[0113] The blood serum can acquire a blood serum from the nonhuman mammal which showed sufficient antibody titer to the antigen used for immunity, and a polyclonal antibody can be acquired by separating and refining this blood serum. As an approach of separating and refining, independent or the approach of combining and processing is mentioned in the chromatography using centrifugal separation, the salting-out by 40 - 50% saturation ammonium sulfate, caprylic-acid precipitate [Antibodies, A Laboratory manual, and Cold SpringHarbor Laboratory (1988)] or a DEAE-sepharose column, an anion-exchange column, protein A, G-column, or a gel filtration column etc.
[0114] (2) Offer the rat which the blood serum showed sufficient antibody titer as a source of supply of an antibody forming cell to the partial fragment polypeptide of the polypeptide of this invention used for the preparation immunity of (Production a) antibody sexuparaous cell of a monoclonal antibody. A spleen will be extracted on three - the 7th, after carrying out the last administration of the antigen matter at the rat which showed this antibody titer.
[0115] Beating of this spleen is carried out in an MEM culture medium (NISSUI PHARMACEUTICAL CO., LTD. make), and it unfolds with pincettes, and supernatant liquid is thrown away after carrying out at-long-intervals alignment separation by 1,200rpm for 5 minutes. After processing the splenic cells of the obtained precipitate fraction for 1 - 2 minutes with the tris-ammonium-chloride buffer solution (pH7.65) and removing an erythrocyte, it washes 3 times by the MEM culture medium, and the obtained splenic cells are used as an antibody forming cell.
[0116] (b) Use the established cell line acquired from the mouse or the rat as a preparation myeloma cell of a myeloma cell. For example, 8-azaguanine resistance mouse (BALB/c origin) myeloma cell stock P3-X63Ag8-U1 [Curr.Topics.Microbiol.Immunol., 81, and 1 (1978), (It abbreviates to P3-U1 hereafter) Europ.J.Immunol., 6, 511 (1976)], SP2 / 0-Ag14 (SP-2) [Nature, 276, and 269 (1978)], P3-X63-Ag8653 (653) [J.Immunol., 123, and 1548] (1979) P3-X63-Ag8 (X63) [Nature, 256, and 495 (1975)] etc. can be used. These cell strains to 8-azaguanine culture-medium [RPMI-1640 culture medium A glutamine (1.5 mmol/l), Although a passage is carried out by culture-medium] which added 8-azaguanine (15microg/(ml)) to the culture medium (henceforth a normal culture medium) which added 2-mercaptoethanol (5x10-5 mol/l), JIENTA mycin (10microg/(ml)), and fetal calf serum (FCS) (CSL company make, 10%) further It cultivates

by the normal culture medium three - four days before cell fusion, and these 2x107 or more cells are used for fusion.
[0117] (c) Throw away supernatant liquid after an MEM culture medium or PBS (1.83g [of phosphoric-acid disodium] and phosphoric-acid 1 potassium 0.21g, 7.65g of salt, 1l. of distilled water, pH7.2) is sufficient, and washing the antibody forming cell acquired by production (b) of a hybridoma, and the myeloma cell acquired by (b), mixing so that the number of cells may be set to antibody forming cell:myeloma cell =5-10:1, and carrying out at-long-intervals alignment separation by 1,200rpm for 5 minutes.
[0118] Unfolding the cell population of the obtained precipitation fraction well, and stirring to this cell population, at 37 degrees C, 0.2-1ml of solutions which mixed per 108 antibody forming cells, polyethylene-glycol-1000(PEG-1000) 2g, MEM 2ml, and dimethyl sulfoxide (DMSO) 0.7ml is added, and 1-2ml of MEM culture media is added several times for for [every] further 1 - 2 minutes.
[0119] After addition, it prepares so that an MEM culture medium may be added and the whole quantity may be set to 50ml. Supernatant liquid is thrown away for this preparation liquid after 5-minute alignment separation at long intervals by 900rpm. After unfolding the cell of the obtained precipitate fraction gently, it depends and absorbs to a measuring pipet, and blows off and appears in it, and it is gently suspended in HAT-medium [culture medium which added hypoxanthine (10-4 mol/l), thymidine (1.5x10-5 mol/l), and aminopterin (4x10-7 mol/l) to normal culture medium] 100ml.
[0120] This suspension is poured distributively 100microl / hole every on the plate for 96 hole culture, and it cultivates for seven - 14 days at 37 degrees C among 5% CO2 incubator. The hybridoma which reacts to the partial fragment polypeptide of the polypeptide of this invention specifically is chosen after culture with the enzyme immunoassay which takes a part of culture supernatant and is stated to anti BODIIZU [Antibodies, A Laboratorymanual, Cold Spring Harbor Laboratory, and Chapter 14 (1988)] etc.
[0121] The following approaches can be mentioned as a concrete example of enzyme immunoassay. The coat of the partial fragment polypeptide of the polypeptide of this invention used for the antigen is carried out to a suitable plate in the case of immunity. The purification antibody obtained by the hybridoma culture supernatant or the below-mentioned (d) is made to react as the first antibody. After making the anti-rat or anti-mouse immunoglobulin antibody which furthermore carried out the indicator with a biotin, an enzyme, the chemiluminescence matter, or a radiation compound as the second antibody react, the reaction according to a marker is performed. What reacts to the polypeptide of this invention specifically is chosen as a hybridoma which produces the monoclonal antibody of this invention.
[0122] The thing repeats cloning twice by limiting dilution, and [uses 1st HT culture medium (culture medium excluding aminopterin from the HAT medium), and uses the 2nd normal culture medium] and in which it was stabilized and strong antibody titer was accepted is chosen as a hybridoma stock which produces the monoclonal antibody of this invention using this hybridoma. (d) Inject intraperitoneal with the 20x106 cell / [the monoclonal antibody production hybridoma cell 5 -] ** to the polypeptide of this invention acquired by (c) to the mouse of eight to 10 weeks old or nude mouse which carried out preparation pristane processing [2, 6, 10, and 14-tetramethyl pentadecane (Pristane) 0.5ml are injected intraperitoneally, and it breeds for two weeks] of a monoclonal antibody. A hybridoma is ascites-tumor-ized in ten - 21 days.
[0123] Ascites is extracted from this ascites-tumor-ized mouse, at-long-intervals alignment separation is carried out by 3,000rpm for 5 minutes, and solid content is removed. A monoclonal antibody can be refined and acquired from the obtained supernatant liquid by the approach used by the polyclonal, and the same approach. The decision of the subclass of an antibody is made using a mouse monoclonal antibody typing kit or a rat monoclonal antibody typing kit. The amount of protein is computed from a Lowry method or the absorbance in 280nm.
[0124] 5. State the method of preparation of the recombination virus vector for producing the polypeptide of this invention in specific human tissue to below the method of preparation of the recombination virus vector which produces the polypeptide of this invention. The DNA fragment of the suitable die length which contains a code part [polypeptide / this] if needed based on

the perfect length cDNA of DNA of this invention is prepared.
[0125] A recombination virus vector is developed by inserting the perfect length cDNA or this DNA fragment in the lower stream of a river of the promotor in a virus vector. In the case of an RNA virus vector, a recombination virus is developed by adjusting a homologous RNA fragment to the DNA fragment of the suitable die length which contains in the perfect length cDNA of DNA of this invention the part which carries out the code of homologous cRNA or this polypeptide, and inserting them in the lower stream of a river of the promotor in a virus vector. An RNA fragment chooses one of the single strands of a sense chain or an antisense strand according to the class of virus vector besides 2 chains. For example, in the case of a Sendai Virus vector, homologous RNA is conversely chosen as an antisense strand for RNA which carries out homologous of the case of a retrovirus vector to a sense chain.
[0126] This recombination virus vector is introduced into the packaging cell which suited this vector. All the cells that can supply the polypeptide to which the recombination virus vector which is missing in at least one of the DNA which carries out the code of the polypeptide which needs a packaging cell for PAKKEJI-NGU of a virus this suffers a loss can be used, for example, can use HEK293 cell of the Homo sapiens kidney origin, mouse fibrocyte NIH3 T3, etc. As a polypeptide supplied in a packaging cell In the case of a retrovirus vector, gag of the mouse retrovirus origin, In the case of a lentivirus vector, polypeptides, such as pol and env, gag of the HIV origin, Polypeptides, such as pol, env, vpr, vpu, vif, tat, rev, and nef, In the case of an adenovirus vector, polypeptides, such as E1A of the adenovirus origin and E1B In the case of an adeno-associated virus, polypeptides, such as Rep (p5, p19, p40) and **** (Cap), are mentioned, and, in the case of Sendai Virus, polypeptides, such as NP, P/C, and L, M, F, HN, are mentioned.
[0127] As a virus vector, it rearranges in the above-mentioned packaging cell, a virus can be produced, and the thing containing a promotor is used for the location which can imprint DNA of this invention by the target cell. As a plasmid vector, MFG [Proc.Natl.Acad.Sci.USA, 92, and 6733-6737 (19 95)], pBabePuro [Nucleic Acids Res., 18, and 3587-3596 (1990)], LL-CG, CL-CG, CS-CG, and CLG [Journal of Virology, 72, and 8150-8157 (1998)], pAdex1 [Nucleic Acids Res., 23, and 3816-3821 (1995)] etc. is used.
[0128] As a promotor, if it can be discovered all over human tissue, all can be used, for example, the promotor of IE (immediateearly) gene of a cytomegalovirus (Homo sapiens CMV), the initial promotor of SV40, the promotor of a retrovirus, a metallothionein promotor, a heat shock protein promotor, SRalpha promotor, etc. can be mentioned. Moreover, the enhancer of Homo sapiens's CMV IE gene may be used with a promotor.
[0129] As a method of introducing the recombination virus vector to a packaging cell, a calcium phosphate method [JP,2-227075,A], the RIPOFE cushion method [Proc.Natl.Acad.Sci.U SA, 84, and 7413 (1987)], etc. can be mentioned, for example.
6. A structural change of the amount of mRNA manifestations of DNA of this invention in a specimen and this mRNA is detectable using DNA of approach this invention which detects the manifestation of DNA of use (1) this invention of DNA of this invention, a polypeptide, or an antibody.
[0130] The organization which acquired from the patient and healthy person who have as a specimen the disease from which manifestation change of DNA of this invention is the cause, Biological materials, such as a blood serum and saliva, the primary culture cell sample which acquired the cell from this biological material and was cultivated in the suitable culture medium in a test tube, Or mRNA or all RNA acquired from what isolated the organization which acquired from the biological material as paraffin or a cryostat intercept is used (this mRNA and all RNA are henceforth called the specimen origin RNA).
[0131] As an approach of detecting, approaches, such as a (1) Northern-blot-technique (2) in situ hybridization method, (3) quantitive PCR method, (4) differential hybridization method [Trends in Genetics 7 and 314 (1991)], (5) DNA-chip method [Genome Research, 6, and 639 (1996)], and the (6) RNase protection assay method, etc. are mentioned, for example. Hereafter, each detecting method is explained in full detail.
[0132] ** Imprint the Northern blot technique specimen origin RNA to base materials, such as a nylon filter, after separation by gel electrophoresis. Hybridization and washing are performed

after an imprint using the indicator probe prepared from DNA of this invention. The band of RNA specifically combined with this probe is detected after washing. By comparing this detection result with a healthy person about the specimen RNA of the patient origin, the amount of manifestations of this RNA and change of structure are detectable. In case hybridization is performed, mRNA made into the purpose under a probe and specimen origin RNA carries out an incubation on the conditions which form a stable hybrid. the approach of an edition [of molecular cloning / 2nd] publication of hybridization and a washing process in order to prevent false positivity -- applying correspondingly -- quantity -- it is desirable to carry out on stringent conditions.

[0133] The indicator probe used for a Northern blot technique can be prepared by making the oligonucleotide which designed the radioisotope, the biotin, the fluorescence radical, the chemiluminescence radical, etc. from the array of DNA of this invention, or this DNA by the well-known approach (nick translation, a random priming, or KINAJINGU), for example incorporate. The amount of association to mRNA of an indicator probe can carry out the quantum of the amount of manifestations of this mRNA by carrying out the quantum of the amount of the united indicator probe from reflecting the amount of manifestations of this mRNA. Moreover, a structural change of this mRNA can be known by analyzing the part on the filter which an indicator probe combines.

[0134] **in Perform hybridization and the process of washing using the specimen which isolated the organization which acquired from the situ hybridization method living body as paraffin or a cryostat intercept, and was obtained, and an indicator probe given in **. The amount of manifestations of mRNA specifically combined with this probe by the same approach as ** is detectable after washing. in the approach indicated by current PUROTO call Inn molecular biology etc. in hybridization and a washing process by the situ hybridization method in order to prevent false positivity -- applying correspondingly -- quantity -- it is desirable to carry out on stringent conditions.

[0135] ** Target RNA is detectable by using the approach based on compounding cDNA using the quantitive PCR method specimen origin RNA, an oligo dT primer or a random primer, and reverse transcriptase (this cDNA is henceforth called the specimen origin cDNA). When the specimen origin RNA is mRNA, any primer of the above-mentioned ** can be used, but when these specimen origins RNA are all RNA, it is required to use an oligo dT primer.

[0136] At the quantitive PCR method, the DNA fragment of the specific mRNA origin is amplified by performing PCR using the primer designed based on the base sequence which makes the specimen origin cDNA a template and DNA of this invention has. Since the amount of this magnification DNA fragment reflects the amount of manifestations of this mRNA, it can carry out the quantum of the amount of this mRNA by placing DNA which carries out the code of an actin, G3 PDH (glyceraldehyde 3-phosphate dehydrogenase), etc. as internal control. Moreover, change of the structure of this mRNA can also be known by separating this magnification DNA fragment by gel electrophoresis. It is desirable to use the suitable primer which amplifies a target sequence specifically and efficiently by this detecting method. Neither association between primers nor association in a primer can be caused, but it can combine with Target cDNA specifically at annealing temperature, and a suitable primer can be designed based on conditions, such as shifting, from Target cDNA on denaturation conditions. The quantum of a magnification DNA fragment needs to carry out to the inside of the PCR reaction which the magnification product is increasing exponentially. Such an PCR reaction can be known by collecting these magnification DNA fragments produced for every reaction, and carrying out quantitative analysis by gel electrophoresis.

[0137] ** Perform hybridization and washing to the base of the filter or slide glass which made DNA of this invention fix, silicon, etc. by using as a probe the specimen origin cDNA prepared by the approach indicated by differential hybridization method and DNA chip method **. Fluctuation of the amount of manifestations of mRNA of this cDNA origin is detectable after washing by measuring the amount of cDNA(s) specifically combined with DNA of this invention. The difference in the manifestation of this mRNA between a contrast specimen and a target specimen is correctly detectable because any approach of a differential hybridization method and

a DNA chip method fixes internal control of an actin, G3 PDH, etc. on a filter or a base. Moreover, indicator cDNA composition can be performed using an indicator dNTP different, respectively based on a contrast specimen and RNA of the target specimen origin, and the quantum of the amount of manifestations of this exact mRNA can be performed by making the filter of one sheet, or the base of one sheet hybridize two indicator cDNA probes to coincidence.

[0138] ** Combine promotor arrays, such as T7 promotor and SP6 promotor, with 3' edge of DNA of RNase protection assay method this invention, and compound the antisense RNA which carried out the indicator using rNTP which carried out the indicator by the imprint system of in vitro using RNA polymerase. After combining this indicator antisense RNA with the specimen origin RNA and making a RNA-RNA hybrid form, it digests by RNase, and a band is made to form by gel electrophoresis and the RNA fragment protected from digestion is detected. By carrying out the quantum of the obtained band, the quantum of the amount of manifestations of mRNA combined with the above-mentioned indicator antisense RNA can be carried out.

[0139] In addition, the DNA fragment obtained from DNA or them which have the base sequence expressed with either of the array numbers 6-10, for example as DNA used for the approach indicated to either ** - ** is mentioned. moreover, as a specimen with which detection by the approach concerned is presented The disease accompanied by activation of unusual immunocytes, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, psoriasis, gout, various encephalomyelitis, The disease accompanied by infection and inflammation of congestive heart failure, traumatic brain injury, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, articular rheumatism, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome), Diseases, such as adult respiratory distress syndrome (ARDS:adultrespiratory distress syndrome), are mentioned, and it can use for a diagnosis of the above-mentioned disease by detecting the manifestation of DNA of this invention by the detection approach concerned.

[0140] (2) Describe how to detect the existence of the variation of DNA of this invention in a test subject, below the approach of detecting the variation of DNA of this invention. The variation of this DNA in a test subject is detectable by comparing directly by DNA and the following approach of this invention. From a test subject, the samples of the primary culture cell origin established from a Homo sapiens biological material or these biological materials, such as an organization, a blood serum, and saliva, are collected, and DNA is extracted out of this biological material or this primary culture cell origin sample (this DNA is hereafter called the specimen origin DNA). Or cDNA is acquired from mRNA of this sample origin with a conventional method (this cDNA is hereafter called the specimen origin cDNA). These specimen origins DNA and cDNA are used as mold, and DNA is amplified by the PCR method etc. using the primer designed based on the base sequence which DNA of this invention has. The obtained magnification DNA is used as a sample DNA.

[0141] The approach of detecting the heteroduplex formed as an approach of detecting whether variation being in Magnification DNA, of hybridization with the DNA strand which has a wild type allele, and the DNA strand which has variation allele can be used. The heteroduplex detecting method according to ** polyacrylamide gel electrophoresis in the approach of detecting a heteroduplex [Trends Genet., 7, and 5 (1991)], ** A single strand conformation polymorphism analysis method [Genomics, 16, and 325-332 (1993)], ** Chemical cleavage method (CCM, chemical cleavage of mismatches) [Human Molecular Genetics (1996) of a mismatch, Tom

Strachan and Andre w P.Read (BIOS Scientific Publishers Li mited)], ** The enzyme-intercept method of a mismatch [Nature Genetics, 9, and 103-104 (1996)], ** Denaturation gel-electrophoresis [Mutat.Res., The approach of 288, a 103-112 (1993)]** protein compaction trial (the protein truncation test:PTT method) [Genomics, 20, and 1-4 (1994)], etc. is mentioned. Hereafter, the above-mentioned approach is explained.

[0142] ** Amplify as a DNA fragment smaller than 200bp by the primer which designed the heteroduplex detecting method specimen origin DNA by polyacrylamide gel electrophoresis, or the specimen origin cDNA to the template based on the base sequence given [this DNA] in either of the array numbers 6-10. 2 chain formation processing by each magnification DNA fragment is performed with a conventional method using DNA of this invention, and this magnification DNA fragment of the test subject origin. Polyacrylamide gel electrophoresis is performed after processing. When a heteroduplex is formed of the variation of this DNA, mobility is later than a gay double strand without variation, and they can be detected as a band different from a gay double strand. It is better for degree of separation to use gels (Hydro-link, MDE, etc.) of special make. If it is retrieval of a fragment smaller than 200bp(s), insertion, deletion, and almost all 1 base substitution are detectable. As for heteroduplex analysis, it is desirable to carry out by the gel of one sheet combined with the single strand conformation polymorphism analysis described below.

[0143] ** Carry out electrophoresis of this DNA amplified as a fragment smaller than 200bp in native polyacrylamide gel after denaturalizing by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template at either of the array numbers 6-10 based on the base sequence of a publication in single strand conformation polymorphism analysis-method single strand conformation polymorphism analysis (SSCP analysis; single strand conformation polymorphism analys is). This amplified DNA is detectable as a band by carrying out the indicator of the primer by radioisotope or the fluorochrome, in case DNA magnification is performed, making this indicator into an index, or carrying out the argentation of the magnification product of a non-indicator after electrophoresis. A fragment with variation is detectable from the difference in mobility by carrying out electrophoresis of the magnification DNA fragment of the DNA origin of this invention, and the thing of the test subject origin to coincidence.

[0144] ** In the chemical cleavage method (the CCM method) of the chemical cleavage method mismatch of a mismatch, one chain of DNA of the location which is carrying out the mismatch by making DNA of this invention hybridize the DNA fragment amplified by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template based on the base sequence given [this DNA] in either of the array numbers 6-10 with the indicator DNA which made the radioisotope or the fluorochrome take in, and processing it with an osmium tetroxide can be made to be able to cut, and variation can be detected. The CCM method is one of the detecting methods sensibility is the highest, and can be adapted also for the specimen of the die length of kilobase.

[0145] ** A mismatch can also be cut in [combining with the T4 phage RIZORU base, the enzyme which participates in restoration of a mismatch by intracellular / like Endonuclease VII /, and RNaseA] enzyme instead of the enzyme-intercept method above-mentioned osmium tetroxide of a mismatch.

** Carry out electrophoresis of the DNA fragment amplified by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template at either of the array numbers 6-10 based on the base sequence of a publication using the gel which has the concentration gradient and temperature gradient of a chemical modifier in denaturation gel-electrophoresis denaturation gel electrophoresis (denaturing gradient gel electrophoresis:DGGE law). The amplified DNA fragment will move in the inside of gel to the location which denaturalizes to a single strand, and after denaturation will not move it. Since the mobility within the gel of DNA amplified in the case where there is nothing with the case where variation is in this DNA differs, it is possible to detect existence of variation. It is good to attach a Pori (G:C) terminal for raising detection sensitivity at each primer.

[0146] ** Protein compaction trial (the protein truncation te st:PTT method)

The phase shift mutation which produces the deficit of a polypeptide by this trial, splice site

mutation, and nonsense mutation are specifically detectable. the special primer which connected T7 promotor array and the eukaryote translational initiation sequence with the five prime end of DNA which has the base sequence expressed with the PTT method to either of the array numbers 6-10 -- designing -- this primer -- using -- the specimen origin RNA -- reverse transcription PCR (RT-PCR) -- cDNA is created by law. A polypeptide will be produced if an in vitro imprint and a translation are performed using this cDNA. When this polypeptide is migrated to gel, the variation which produces a deficit does not exist if it is in the location where the migration location of this polypeptide is equivalent to a perfect length polypeptide, but a deficit is in this polypeptide, this polypeptide can migrate in a location shorter than a perfect length polypeptide, and extent of a deficit can be known from this location.

[0147] When variation is detected by the above-mentioned approach, it is possible to determine the base sequence of the specimen origin DNA which has variation with a conventional method, and the specimen origin cDNA using the primer designed based on the base sequence which DNA of this invention has. In the case of the test subject in whom the specimen origin DNA or the specimen origin cDNA has a specific disease, the variation leading to this disease can be specified by analyzing the determined base sequence. Henceforth, it can use for a diagnosis of a disease by detecting this variation.

[0148] In detection of variation other than the variation in the coding region of DNA detected by the above-mentioned approach, it can detect by inspecting the intron near this DNA and in this DNA, and a non-coding region like a regulatory sequence. The disease resulting from the variation in a non-coding region can be checked by detecting the unusual size in the disease patient at the time of comparing with a contrast specimen according to the approach indicated above, or mRNA of an unusual volume.

[0149] Thus, about this DNA existence of the variation in a non-coding region was suggested saying, it can clone by using for either of the array numbers 6-10 DNA which has the base sequence of a publication as a probe of hybridization. It can search for the variation in a non-coding region according to one of above-mentioned approaches.

[0150] The found-out variation can be identified as SNPs (single nucleotide poly mol FIZUMU) with a chain with a disease by performing statistics processing according to the approach indicated by Handbook of Human Genetics Linkage.The John Hop kins University Press and Baltimore (1994). As a diagnosable test subject, by the approach of detecting the above-mentioned variation The disease accompanied by activation of unusual immunocytes, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, psoriasis, gout, various encephalomyelitis, The disease accompanied by infection and inflammation of congestive heart failure, traumatic brain injury, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, articular rheumatism, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemicinflammatory response syndrome), Those who have ones, such as adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome), of diseases can be mentioned.

[0151] (3) The approach antisense RNA / DNA technical [bioscience and the industry which control the imprint or translation of DNA which carries out the code of the polypeptide of this invention using DNA or the oligonucleotide of this invention, and 50,322 (1992), Chemistry, 46, 681 (1991), Biotechnology, 9, and 358 (1992), Trends in Biotechnology, 10, and 87 (1992), Trends in Biotechnology, 10, and 152 (1992), With a cell technology, 16, 1463 (1997)], a triple helix technique [Trends in Biotechnology, 10, and 132 (1992)], etc. The imprint or translation of DNA

which carries out the code of the polypeptide of this invention can be controlled using DNA of this invention. For example, the system (a living body is included) which can discover the polypeptide of this invention for DNA or the oligonucleotide of this invention is made to live together, and the manifestation of this polypeptide can be controlled on an imprint and translation level.

[0152] This control approach Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, traumatic brain injury, The disease accompanied by infection and inflammation of inflammatory bowel disease etc., a Burkitt lymphoma, Hodgkin's disease, The disease accompanied by unusual cell proliferations, such as various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as articular rheumatism and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic in flammatory response syndrome), The variation of DNA which carries out the code of the polypeptide of this invention can use adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc. for the therapy or prevention of a disease used as a cause.

[0153] (4) It is possible to acquire the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of this invention by the well-known approach [the volume the 2nd edition of molecular cloning and for University of Tokyo Institute of Medical Science carcinostatic research sections, a new cell technology experiment protocol, and Shujunsha (1993)], using as a probe DNA or the oligonucleotide of approach this invention which acquires the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of this invention using DNA or the oligonucleotide of this invention. For example, the thing of a rat or the Homo sapiens origin is acquirable by the following approaches.

[0154] It screens by approaches, such as plaque hybridization, to the genomic DNA library produced using the chromosome DNA isolated from cell and organization of a rat or Homo sapiens by using DNA or the oligonucleotide (especially 5' of cDNA near part) of this invention as a probe. The genomic DNA to hybridize is acquired by this screening. Promoterregion and imprint regulatory region can be obtained from this DNA. Moreover, an exon / intron structure can be clarified by comparing the base sequence of genomic DNA and the base sequence of cDNA which were acquired.

[0155] In addition, also in other nonhuman mammals, the promoterregion and the imprint regulatory region of this DNA are acquirable using the same approach. The field which participates in the basic imprint of DNA which carries out the code of the polypeptide of this invention in a mammalian cell as promoterregion is mentioned, and a field including an enhancer sequence, a silencer array which decreases which reinforces the basic imprint of DNA which carries out the code of the polypeptide of this invention as imprint regulatory region is mentioned. For example, the promoterregion and the imprint regulatory region which participate in the imprint of DNA which carries out the code of the polypeptide of this invention by human bone marrow can be mentioned. The promotor and imprint regulatory region which were obtained are applicable to the below-mentioned screening approach, and also they are useful in order to analyze the controlling mechanism of an imprint of this DNA.

[0156] (5) Various test compounds can be added to the cell strain of the approach patient origin which acquires the physic which controls the imprint of this DNA by screening using DNA which carries out the code of the polypeptide of this invention, and the matter which controls or promotes an imprint or translation of this DNA can be screened by authorizing the change in the manifestation of mRNA using DNA of this invention. The change in the manifestation of mRNA of

this DNA is detectable by the above-mentioned PCR method and the above-mentioned Northern blot technique, and the RNase protection assay method.

[0157] Various test compounds can be added to a patient origin cell strain, and the matter which promotes an imprint or translation of this DNA can be screened by authorizing the change in the manifestation of this polypeptide using the antibody which recognizes the polypeptide of this invention specifically. The change in the manifestation of this polypeptide is detectable by immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as the above-mentioned fluorescent antibody technique, enzyme immunoassay (the ELISA method), radioactive substance indicator immunity antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, the western blotting method, the dot blotting method, the immunoprecipitation method, and the sandwiches ELISA method.

[0158] The polypeptide of this invention on moreover, the lower stream of a river of the promoter region of DNA which carries out a code, and imprint regulatory region The reporter plasmid which connected the chloramphenicol acetyltransferase (CAT) gene and the luciferase gene as a reporter gene is built. After introducing into a suitable cell host and obtaining a transformant, the physic which controls by imprint level the manifestation of DNA which carries out the code of the polypeptide of this invention can be screened by adding various examined substances to the transformant, and analyzing the change in the manifestation of a reporter gene.

[0159] (6) How to acquire the physic which acts on the polypeptide of this invention by the screening approach using the polypeptide of this invention.

The physic which acts on the polypeptide of this invention can be screened by making the transformant which discovered the polypeptide of this invention, or the partial peptide of this polypeptide, and various examined substances live together, and analyzing fluctuation of activation of NF-kappa B in this transformant. Moreover, it can use for the medicinal screening to which the partial peptide of this refined polypeptide or this polypeptide also acts on this polypeptide specifically. The matter obtained by this screening is useful as physic for the therapy of the disease in which DNA and the polypeptide of this invention participated.

[0160] Hereafter, two sorts of screening procedures are explained.

Screening procedure (1)

The microorganism which carried out the transformation so that the polypeptide of this invention or the partial peptide of this polypeptide might be produced, an animal cell or an insect cell (the transformant for retrieval is called henceforth), and an examined substance are made to live together in an aquosity medium. According to the approach of a publication, the activity of NF-kappa B is measured after coexistence to above-mentioned 2. Microorganism, animal cell, or insect cell of the host who has not done a transformation is compared as a control group, and the target matter can be acquired by choosing the examined substance which fluctuates extent of activation of NF-kappa B in this transformant. Moreover, it can make into an index to check association to this transformant for retrieval of the compound specifically combined with this transformant for retrieval, or a polypeptide, and contention screening of the target compound can be carried out by the same approach as the above.

[0161] The polypeptide which constitutes a part of polypeptide of refined this invention or this polypeptide can be used for choosing the target compound specifically combined with this polypeptide. In order to carry out the quantum of the target compound, the polypeptide of this invention can be performed by the above-mentioned immunologic procedure using the antibody recognized specifically. Moreover, contention screening of the target compound can be carried out for checking association of the target compound combined with the polypeptide of this polypeptide or this polypeptide at an index.

[0162] Screening procedure (2)

Many peptides which constitute this a part of polypeptide can be compounded to high density on a plastics pin or a solid-state base material of a certain kind, and the compound or polypeptide alternatively combined with this peptide can be screened efficiently (WO 84/03564). In addition, the gene which receives transcriptional control by the polypeptide of this invention can be screened by analyzing gene expression using the transformant which discovers the polypeptide

of this invention.
[0163] (7) The gene therapy agent using the virus vector containing RNA which consists of DNA of this invention, DNA of gene therapy agent this invention containing RNA which consists of this DNA and a homologous array or this DNA, and a homologous array can be manufactured by preparing the basis which was produced by above-mentioned 5. and which is rearranged and is used for a virus vector and a gene therapy agent [Nat ure Genet., 8, and 42 (1994)]. If it is the basis usually used for injections as a basis used for a gene therapy agent, what kind of thing may be used and the mixed solution of amino acid solutions, such as sugar solutions, such as salting in liquid, such as mixture of distilled water, a sodium chloride or a sodium chloride, and mineral salt, a mannitol, a lactose, a dextran, and a glucose, a glycine, and an arginine, an organic-acid solution or salting in liquid, and a glucose solution etc. will be raised. Moreover, according to a conventional method, assistants, such as surfactants, such as vegetable oil, such as an osmotic-pressure regulator, pH regulator, sesame oil, and soybean oil, lecithin, or a nonionic surface active agent, may be used for these bases, and injections may be prepared as a solution, suspension, and dispersion liquid. these injections -- actuation of disintegration, freeze drying, etc. -- business -- the time -- as the pharmaceutical preparation for the dissolution -- it can also prepare . In the case of a liquid, the gene therapy agent of this invention remains as it is, and in the case of an individual, it can dissolve in the above-mentioned basis which carried out sterilization processing as occasion demands just before gene therapy, and can be used for a therapy. As a medication method of the gene therapy agent of this invention, the approach of prescribing for the patient locally can be raised so that it may be absorbed by a patient's therapy part.
[0164] A virus vector can be prepared by combining with an adenovirus vector the complex which produced complex combining the specific poly lysine-conjugate antibody in adenovirus hexone protein, and was obtained in DNA of suitable this invention of size. Stability is reached at a target cell, and it is incorporated by intracellular by endosome, and is decomposed by intracellular, and this virus vector can make DNA discover efficiently.
[0165] (-) The virus vector which used as the base Sendai Virus which is a chain RNA virus is also developed (Japanese Patent Application No. 9-517213, Japanese Patent Application No. 9-517214), and the Sendai Virus vector which incorporated KRGF-1 gene for the purpose of gene therapy can be produced. This DNA can be conveyed to the focus also by the non-virogene importing method.
[0166] By the well-known non-virogene importing method, in the field concerned A calcium phosphate coprecipitation method [Virology, 52, 456-467;(1973) Science, 209, and 1414-1422 (1980)], Microinjection method [Proc. Natl.Acad.Sci.USA, 77 and 5399-5403 1980 ;P roc.Natl.Acad.Sci.USA, 77, 7380-7384;(1980) Cell, 27, 223-231;(1981) Nature, 294, and 92-94 (1981) --] -- Liposome Minded membrane fusion-mediation importing method [Proc.Natl. Acad.Sci.USA and 84, 7413-7417;(1987) Biochemistry, 28, 9508-9514;(1989) J.Biol.Chem., 264, and 12126-12129;(1989) Hum.Gene T her. and 3,267-275 () 1992;Science and 249, Method [of 1285-1288;(1990) Circulation, 83 2007-2011 (1992)] or direct DNA incorporating, and acceptor-medium DNA importing] [Science, 247, and 1465-1468 ;J.Biol.Chem., (1990) 266 14338-14342 (1991) ;P roc.Natl.Acad.Sci.USA, 87, 3655-3659;(1991) J.Biol.Chem., 26 4 and 16985-16987 ; BioTechniques, (1989) 11 474-485 (1991) ;P roc. Natl.Acad.Sci.USA, 87 3410-3414 (1990) ;P roc. Natl.Acad.Sci.USA, 88 4255-4259 (1991) ;P roc. Natl.Acad.Sci.USA, 87 4033-4037 (1990) ;P roc.Natl.Acad.Sci.USA, 88, 8850-8854;(1991) Hum. Gene Ther., 3, 147-154(1991)], etc. can be mentioned.
[0167] By the membrane fusion-mediation importing method through liposome, it is reported in the research on a neoplasm by medicating with a liposome preparation object directly the organization which considers as a target that incorporation and manifestation of the organization concerned of a local gene are possible [Hum.Gene Ther., 3, and 399-410 (1992)]. Therefore, the same effectiveness is expected also by the disease focus in which DNA and the polypeptide of this invention participate. In order to carry out direct targetting of the DNA to the focus, a direct DNA incorporation technique is desirable. Acceptor-medium DNA import is performed for example, through the poly lysine by carrying out conjugate of the DNA (the gestalt of the

supercoiling plasmid which usually carried out the ring closure in share being taken) to polypeptide ligand. Ligand is chosen based on existence of the ligand acceptor to which it corresponds on a target cell or the cell surface of an organization. By request, a blood vessel can be directly injected with the ligand-DNA conjugate concerned, and it can point to it in the target tissue to which internalization of acceptor association and DNA-protein complex takes place. In order to prevent intracellular destruction of DNA, concurrent infection of the adenovirus can be carried out and an endosome function can also be collapsed.
[0168] (8) The organization containing the polypeptide or this polypeptide of this invention is immunologically detectable by making an antigen-antibody reaction perform using the antibody which recognizes specifically the polypeptide of approach this invention which detects the polypeptide of this invention immunologically using the antibody of this invention. This detecting method Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, traumatic brain injury, hypertrophic arthritis, psoriasis, gout, various encephalomyelitis, The disease, Burkitt lymphoma accompanied by infection and inflammation of congestive heart failure, inflammatory bowel disease, etc., The disease accompanied by unusual cell proliferations, such as Hodgkin's disease, various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as rheumatoid arthritis and fibroid lung, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome), The variation of DNA which carries out the code of the polypeptide of this invention can use adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc. for a diagnosis of the disease used as a cause. Moreover, this detection approach is used also for the quantum of a polypeptide.
[0169] as detection and an approach of carrying out a quantum, immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as a fluorescent antibody technique, enzyme immunoassay (the ELISA method), radioactive substance indicator immunity antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, a western blotting method, the dot blotting method, an immunoprecipitation method, the sandwiches ELISA method [a monoclonal antibody experiment manual (Kodansha -- scientific) (1987), New Biochemistry Experiment Lectures 5, and an immunobiochemistry approach (Tokyo Kagaku Dojin) (1986)], etc. are mentioned immunologically.
[0170] After a fluorescent antibody technique makes the antibody of this invention react to the microorganism, the animal cell, insect cell, or organization which discovered the polypeptide of this invention out of intracellular or a cell and makes the anti-mouse IgG antibody which carried out the label with fluorescent materials, such as fluorescin isothiocyanate (FITC), further, or its fragment react, it is the approach of measuring a fluorochrome with flow cytometer.
[0171] Enzyme immunoassay (the ELISA method) is the approach of measuring coloring coloring matter with an absorptiometer, after making the anti-mouse IgG antibody which the antibody of this invention was made to react to the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out of intracellular or a cell, and gave it enzyme labeling, such as a peroxidase and a biotin, etc. further, or a joint fragment react.
[0172] Radioactive substance indicator immunity antibody technique (RIA) is the approach of measuring with a scintillation counter etc., after making the anti-mouse IgG antibody which the antibody of this invention was made to react to the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out of intracellular or a cell, and gave it the radiation indicator further, or its fragment react. After an immunocyte staining technique and an immunity staining method make the antibody which recognizes this polypeptide specifically in the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out

of intracellular or a cell react and make the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescent materials, such as FITC, a peroxidase, and a biotin, further, or its fragment react, they are the approach of observing using a microscope.

[0173] The microorganism which discovered this polypeptide out of intracellular or a cell with the western blotting method, After carrying out fractionation of an animal cell, an insect cell, or the extract of an organization by SDS-polyacrylamide gel electrophoresis [Antibodies-A Laboratory Manual and Cold SpringHarbor Laboratory (1988)], Blotting of this gel is carried out to the PVDF film or a nitrocellulose membrane. After making the antibody which recognizes this polypeptide of this invention specifically react to this film and making the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescent materials, such as FITC, a peroxidase, and a biotin, further, or its fragment react, it is the approach of checking.

[0174] After the dot blotting method carries out blotting of the microorganism which discovered this polypeptide out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization to a nitrocellulose membrane, makes the antibody of this invention react to this film and makes the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescent materials, such as FITC, a peroxidase, and a biotin, further, or a joint fragment react, it is the approach of checking.

[0175] An immunoprecipitation method is an approach of adding the support which has a specific binding affinity to immunoglobulins, such as protein G-sepharose, and making an antigen antibody complex sedimenting, after making the microorganism which discovered the polypeptide of this invention out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization react with the antibody which recognizes this polypeptide specifically.

[0176] The sandwiches ELISA method is the antibody which recognizes the polypeptide of this invention specifically. The antibody which is one side beforehand among two kinds of antibodies from which an antigen recognition site differs is made to stick to a plate. The indicator of another antibody is carried out with enzymes, such as fluorescent materials, such as FITC, a peroxidase, and a biotin. After making the microorganism which discovered this polypeptide out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization react to an antibody adsorption plate, it is the approach of making the antibody which carried out the indicator reacting and performing the reaction according to a marker.

[0177] (9) It is useful to identify a structural change of the polypeptide which has changed and discovered the amount of manifestations of this polypeptide in the approach Homo sapiens biological material row Homo sapiens primary culture cell which diagnoses a disease using the antibody which recognizes the polypeptide of this invention specifically, when getting to know the danger of showing the symptoms of a disease in the future, and the cause of a disease whose symptoms were already shown. As an approach of detecting and diagnosing the amount of manifestations of this polypeptide, and a structural change, immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as the above-mentioned fluorescent antibody technique and the above-mentioned enzyme immunoassay (the ELISA method), radioactive substance indicator immunity antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, a western blotting method, the dot blotting method, an immunoprecipitation method, the sandwiches ELISA method, etc. are mentioned.

[0178] As a specimen with which the diagnosis by the above-mentioned approach is presented, allergy, atopy, The disease accompanied by activation of unusual immunocytes, such as asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, traumatic brain injury, hypertrophic arthritis, The disease accompanied by infection and inflammation of psoriasis, gout, various encephalomyelitis, congestive heart failure, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, rheumatoid arthritis, and fibroid lung, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on

the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome), Adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc., The cell and cell extract which were acquired from the biological material itself or these biological materials, such as the organization and blood which were acquired from the patient of the disease from which the variation of DNA which carries out the code of the polypeptide of this invention is the cause, a blood serum, urine, facilities, and saliva, are used. Moreover, what isolated the organization which acquired from the biological material as paraffin or a cryostat intercept can also be used.

[0179] The ELISA method and a fluorescent antibody technique using a microtiter plate as an approach of detecting immunologically, a Western blot technique, an immunity staining method, etc. are mentioned. The radioimmunoassay method using the antibody which recognizes the polypeptide of this invention and the polypeptide of this invention which carried out the indicator with radioisotopes using two kinds of monoclonal antibodies from which an epitope differs in the liquid phase as an approach of carrying out a quantum immunologically among the polypeptide of this invention and the antibody which reacts, such as the sandwiches ELISA method and 125I, etc. is mentioned.

[0180] (10) Use the recombination vector which comes to contain DNA of production this invention of a knock out nonhuman animal using DNA of this invention. In embryonic stem cells (embryonic stem cell), such as the target nonhuman animal, for example, a cow, a sheep, a goat, Buta, a horse, a mouse, and a fowl DNA which carries out the code of the polypeptide of this invention on a chromosome -- the technique of well-known homologous recombination -- [-- for example The variation clone permuted by the array of inactivation or arbitration by] (1987), such as Nature, 326, 295 (1987), Cell, 51, and 503, is produced ([Nature, 350, and 243] (1991)). [for example,] The chimera individual which consists of an embryonic stem cell clone and a normal cell can be prepared using the variation clone of an embryonic stem cell by technique, such as the impregnation chimera method to the blastocyst (blastcyst) of the fertilized egg of an animal, or the set chimera method. The individual which has the variation of arbitration by crossing of this chimera individual and a normal individual in DNA which carries out the code of the polypeptide of this invention on the chromosome of the cell of the whole body can be obtained, and the manifestation of DNA which carries out the code of the polypeptide of this invention can obtain a knock out nonhuman animal as a part or an individual controlled completely out of the gay individual by which variation went into the both sides of homologue by crossing of that individual further.

[0181] Moreover, it is also possible to produce a knock out nonhuman animal by introducing variation to the location of the arbitration of DNA which carries out the code of the polypeptide of this invention on a chromosome. For example, it is possible to also make the activity of the product change by a permutation, deletion, insertion, etc. carrying out a base all over the translation field of DNA which carries out the code of the polypeptide of this invention on a chromosome, and introducing variation. Moreover, it is possible by introducing the same variation to the manifestation regulatory region to also make extent of a manifestation, a stage, tissue specificity, etc. change. It is also still more possible to control a manifestation stage, a manifestation part, the amount of manifestations, etc. by combination with a Cre-loxP system more positively. the example [Cell, 87, and 131 7 (1996)] to which deletion of the purpose gene was carried out only in the field using the promoter discovered in a specific field with a brain as such an example, and the adenovirus which discovers Cre -- using -- the target stage -- an organ -- the example [Science, 278, and 5335 (1997)] to which deletion of the purpose gene was carried out specifically is known.

[0182] Therefore, the knock out nonhuman animal which can control a manifestation by the stage and organization of arbitration, or has insertion of arbitration, deletion, and a permutation in the translation field and manifestation regulatory region in this way also about DNA which carries out the code of the polypeptide of this invention on a chromosome is producible. A knock out nonhuman animal can guide the symptom of the various diseases resulting from the

polypeptide of this invention by the stage of arbitration, extent of arbitration, or the part of arbitration. Thus, the knock out nonhuman animal of this invention serves as very useful animal model in the therapy and prevention of various diseases resulting from the polypeptide of this invention. It is very useful especially as models for evaluation, such as the remedy, a prophylactic and functional food, and health food.

[0183] 7. As an approach of introducing variation into the variation installation this polypeptide of the polypeptide of variation installation of the polypeptide of this invention, and selection (1) this invention of a functional alteration variant, what kind of approach of deletion, insertion, and a permutation may be used. The deletion and insertion of a polypeptide are possible by carrying out deletion of this DNA fragment by the approach indicated by the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, etc. in DNA which carries out the code of this polypeptide, or making a suitable DNA fragment insert.

[0184] For example, it can obtain by graduating by DNA polymerase, such as Klenow Fragment (product made from TaKaRa), and making it re-connect after digestion, with this restriction enzyme of marketing of the plasmid which included a the same and different restriction enzyme site suitable in this DNA for a two-piece header and this DNA when it was a deletion mutant, if it is a flush end, if it is a cohesive end as it is. If it is an insertion variant, it can obtain by making double stranded DNA suitable after flush-end-izing insert and connect. A permutation variant is Error Prone as an approach of introducing variation at random. The PCR method [Trends In Biotechnology, 16, and 76 (1998)] etc. can be used. As an approach of introducing variation into the target location, the PCR method [Mutagenesis and Synthes is of Novel Recombinant Genes Using PCR, PCR PRIMER A LABORATORY MANUAL, 603 (1994)] or QuikChangeTMSite-Directed Mutagenesis Kit (product made from STRATAGENE) using a primer with variation etc. can be used.

[0185] (2) Selection of an activity rise alteration variant [as opposed to NF-kappa B activation according to the approach indicated to above-mentioned 2.] is more possible than the variant of this polypeptide produced by selection (1) of the functional alteration variant of the polypeptide of this invention. The functional alteration variant which went up the NF-kappa B activation function can be obtained by introducing each of the variant of this polypeptide and this polypeptide into a reporter cell, and specifically choosing the variant which raised reporter activity from this polypeptide. Moreover, a dominant negative variant can be obtained by choosing the variant of this polypeptide that controls NF-kappa B activation under the stimulus existence which activates NF-kappa B.

[0186] The variant of this polypeptide is introduced into a reporter cell, and, specifically, it is cytokine (TNF-alpha). T cell mitogen, such as TNF-beta, IL-1alpha, IL-1beta, IL-2, and LIF (an antigen stimulus) Lectin, an anti-T cell receptor antibody, anti-CD2 antibody, anti-CD3 antibody, anti-CD28 antibody, calcium ionophore, and B cell mitogen (an anti-IgM antibody --) anti-CD40, leukotriene, LPS and PMA, a parasitism somesthesis stain, virus infection (it CMV(s) HIV-1, HTLV-1, and HBV and EBV --) HSV-1, HHV-6, NDV, Sendai Virus, adenovirus, etc., A virus product (double stranded RNA, Tax and HBX, EBNA-2, LMP-1 grade), DNA destructive matter and protein synthesis inhibitor (for example, cycloheximide) A dominant negative variant can be obtained by giving the stimulus which activates NF-kappa B, such as ultraviolet rays, a radiation, and oxidation stress, and choosing the variant of this polypeptide which fell rather than the time of reporter activity having not introduced the variant.

[0187] In addition, the obtained dominant negative variant (Dominant Negative mutants; dominant functional control variant) can be applied to inflammation response control or growth control of a malignant cell, and may be able to use for the gene therapy of the disease accompanied by activation of NF-kappa B DNA which carries out the code of this dominant negative variant. An example is raised to below and this invention is explained concretely. However, these examples are the things for explanation and do not restrict the technical range of this invention.

[0188]

[Example] From the [example 1] Homo sapiens large intestine, the large intestine of the production Homo sapiens of a Homo sapiens fat tissue origin perfect length cDNA library, and fat tissue, mRNA was extracted [edition / 2nd / of molecular cloning] by the approach of a

publication. Furthermore, polyA+RNA was refined by oligo dT cellulose. The cDNA library was produced from each polyA+RNA with Oligo-capping method [Gene, 138, and 171-174 (1994)]. According to the approach of a publication, composition of BAP (Bacterial A lkaline Phosphatase) processing, TAP (Tobacco Acid Phosphatase) processing, RNA ligation, and the first chain cDNA and removal of RNA were performed to a protein nucleic-acid enzyme, 41, 197-201 or (1996) Gene, 200, and 149-156 (1997) using Oligo-cap linker (array number 11) and Oligo dT primer (array number 12). The double strand cDNA was amplified by having used the first obtained chain cDNA as mold by PCR using two sorts of primers, the sense primer by the side of a five prime end (array number 13), and the antisense primer by the side of a three-dash terminal (array number 14), and it cut by SfiI. The commercial kit:GeneAmp XL PCR kit (product made from Perkin Elmer) was used, for 1 minute was repeated at 95 degrees C after heat treatment for 5 minutes, it repeated [95 degrees C] the reaction cycle for 10 minutes 12 times for 1 minute and at 72 degrees C by 58 degrees C, and PCR performed it by holding at 4 degrees C after that.

[0189] The above-mentioned magnification cDNA was inserted in vector pME18SFL3 (GeneBank AB [009864], an expression vector, 3392bp) cut by DraIII, and the cDNA library was produced. About the plasmid DNA of each of the obtained clone, the base sequence of 5 'edge and 3' edge of cDNA DNA sequencing reagent () [Dye Terminator] Cycle SequencingFS Re ady Reaction Kit and dRhodamine Terminator Cycle Sequencing FS ReadyR eaction Kit or BigDye Terminator Cycle Sequencing FS Ready ReactionKit, and the product made from PE Biosystems are used. After performing a sequence reaction according to a manual, the base sequence was determined using the DNA sequencer (ABI PRISM 377, product made from PE Biosystems).

[0190] The artificial promotor who repeated the NF-kappa B recognition sequence in establishment IFN-beta of the reporter cell strain by which manifestation control of the luciferase activity is carried out by the [example 2] NF-kappa B enhancer (array number 15) 3 times was produced, and it inserted in 5' upstream region of the luciferase gene of a luciferase reporter vector (pAGE-luc; JP,3-22979,A, the experimental medicine, 7, and 96-103 (1989)) (it is henceforth called pIF-luc). This plasmid 4microg was dissolved in TE buffer solution [10 mmol/l tris-HCl (pH8.0), 1 mmol/l EDTA (ethylenediaminetetraacetic acid sodium)] so that it might be set to 1micro g/mu l, and transgenics was carried out to the Homo sapiens nephrocyte stock 293 (product made from Clontech) 1.6x106 piece by the electroporation method (the product made from BIO-RAD: Gene PulserTM). pIF-luc contains the hygromycin (Hygromycin) resistance gene, and after transgenics established the stabilization transformant for culture and hygromycin as a selective marker of transgenics by the RPMI culture medium [RPMI1640 (Nippon Suisan Kaisha, Ltd. make), 10% calf blood serum, 0.05 mmol/l-mercaptoethanol, 25 U/ml penicillin G, and 25U/ml streptomycin] which added hygromycin 0.2 g/l. Among stabilization transformant, by TNF-alpha stimulus, the stock which guided the high luciferase activity of 670 times as compared with no stimulating was chosen (it is henceforth called 293-/IF-LUC), and it used for the following manifestation assays.

[0191] Shaking culture of the clone which determined the base sequence in the analysis example 1 over NF-kappa B activation of the perfect length DNA using [example 3] 293 / IF-LUC was respectively carried out at 37 degrees C for 16 hours among 2ml (Yeast ex tract 10 g/l, Trypton 16 g/l, NaC 5 g/l) of 2xYT culture media which added ampicillin (100 mg/l). The centrifugal separator recovered the fungus body after culture, and the plasmid was respectively prepared by the approach of attachment data using the plasmid preparation kit (QIAPrep96 Turbo Miniprep Kit, product made from QIAGEN). It poured distributively so that it might become a plate with 20,000 per one well about 293 / IF-LUC cell 96 well, and it cultivated in the CO2 incubator at 37 degrees C for 16 hours. The RIPOFE cushion reagent (LIPOFECT AMINE 2000TM Reagent, product made from GIBCO BRL) was used for this cultured cell, respectively, and the 0.25micro of the above-mentioned plasmid abbreviation g was introduced into it according to the approach of attachment data. It used at 37 degrees C for 16 hours, a luciferase activity measurement reagent (LucLiteTM, product made from Packar) and luciferase activity measurement equipment (ARVO 1420 MULTILABEL COUNTER, product made from WALLC) were used after culture in the CO2 incubator, and luciferase activity was measured.

[0192] Consequently, COL03279 (DNA clone which has the base sequence of the array number 6), COL06772 (DNA clone which has the base sequence of the array number 7), ADKA01604 (DNA clone which has the base sequence of the array number 8), [when the plasmid of each clone of ADSU00701 (DNA clone which has the base sequence of the array number 9), and CAS01989 (DNA clone which has the base sequence of the array number 10) is introduced] As compared with negative control (pME18SFL3 is used), one 12.5 times, 6.3 times, 4.4 times, 2.7 times, and 3.0 times the activity of this was checked, respectively. DNA of this invention was respectively acquired from this clone.
[0193] the quantum of the amount of manifestations in the various organs of DNA of this invention accepted in each clone of the detection COL03279, COL06772, ADKA01604, and ADSU00701 of the amount of manifestations in the various organs of DNA of [example 4] this invention -- a law -- according to the method [PCR Protocols, Academic Press (1990), etc.], it carried out as follows using the half-quantitive PCR method. Moreover, the quantum of the transcript of the glyceraldehyde 3-phosphate dehydrogenase (glyceraldehyde-3-phosphate dehydrogenase;G3 PDH) considered to carry out the comparable manifestation in every cell was performed to coincidence, and it checked that it was practically equal to the conversion efficiency to a single strand cDNA from mRNA by the difference in the amount of mRNA(s) between cells, and the reverse transcriptase between samples.
[0194] mRNA of the Homo sapiens organ origin (the product made from Clontech: 3 caudate nucleus 2 brain 1 suprarenal gland) Four hippocampi, 5 substantia nigra, six thalami, the 7 kidney, the 8 pancreas, nine hypophyses, ten small intestines, Eleven bone marrow, 12 amygdalas, 13 cerebellums, 14 corpus callosa, 15 embryo brain, 16 embryo kidney, 17 embryo liver, 18 embryo lungs, the 19 heart, 20 liver, 21 lungs, 22 lymph gland, 23 mammary glands, 24 placentas, 25 prostate glands, 26 salivary glands, 27 skeletal muscle, 28 spines, The single strand cDNA was compounded using the cDNA composition kit (product made from SUPERSCRIPTTM Preamplification System; BRL) from 29 spleens, the 30 stomach, 31 testes, 32 thymus glands, the 33 thyroid, 34 tracheae, and 35 uteri. The single strand cDNA was compounded from mRNA of 1microg, and it diluted 240 times with water, and was used as mold of PCR. The synthetic DNA of a publication was used for the array numbers 16 and 17 based on the base sequence information from COL03279, the array numbers 18 and 19 based on the base sequence information from COL06772, the array numbers 20 and 21 based on the base sequence information from ADKA01604, and the array numbers 22 and 23 based on the base sequence information from ADSU00701 as a primer for PCR. The PCR reaction was performed according to the description using 10xGene Taq Universal Buffer and 2.5 mmol/ldNTP Mixture of NIPPON GENE Recombinant Taq DNA Polymerase (GeneTaq) and attachment. Thermal SAIKURA made from MJ RESERCH is used, and it is [degrees C / 94] 26 - 30 cycle ****** about the reaction for 2 minutes for 1 minute and at 72 degrees C for 30 seconds and in 60 degrees C. Reaction mixture was analyzed by agarose gel electrophoresis and ethidium-bromide dyeing.
[0195] A result is shown in drawing 1 -4. DNA of this invention accepted in each clone of COL03279, COL06772, ADKA01604, and ADSU00701 had discovered the difference of strength by each clone and each organ by all 35 which a certain thing examined sorts of organs.
[0196]
[Effect of the Invention] According to this invention, allergy, atopy, asthma, pollinosis, respiratory tract irritation, The disease accompanied by activation of unusual immunocytes, such as an autoimmune disease and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, traumatic brain injury, psoriasis, The disease accompanied by infection and inflammation of gout, various encephalomyelitis, congestive heart failure, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, rheumatoid arthritis, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve

cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:syste mic inflammatory responsesyndrome), Retrieval of remedies, such as adult respiratory distress syndrome (ARDS:adult respiratorydistress syndrome), The antisense DNA/RNA of DNA and this DNA which carries out the code of a useful polypeptide and this polypeptide to development, The antibody which recognizes the gene therapy using this DNA and this polypeptide, the activity rise alteration object of this polypeptide, the dominant negative variants of this polypeptide, and these directions can be offered.
[0197]
[Array table free text]
Explanation of an array number 11-artificial array: Composition RNA (oligo cap linker array)
Explanation of an array number 12-artificial array: Synthetic DNA (oligo dT primer array)
Explanation of an array number 13-artificial array: Synthetic DNA (sense primer array by the side of a five prime end)
Explanation of an array number 14-artificial array: Synthetic DNA (antisense primer array by the side of a three-dash terminal)
Explanation of an array number 15-artificial array (transcription factor NF-kappa junction sequence)
Explanation of an array number 16-artificial array: Synthetic DNA (synthetic primer array which considered organization manifestation distribution)
explanation: of an array number 17-artificial array -- explanation: of a synthetic DNA array number 18-artificial array -- explanation [of a synthetic DNA array number 19-artificial array]: -- explanation [of a synthetic DNA array number 20-artificial array]: -- explanation [of a synthetic DNA array number 21-artificial array]: -- explanation [of a synthetic DNA array number 22-artificial array]: -- explanation [of a synthetic DNA array number 23-artificial array]: -- a synthetic DNA [0198]
[Layout Table]
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LysLeu Cys Ser Ile Cys Lys Ala Met 180 185190 Glu Thr Trp Leu Ser Ala Asp Pro GlnHis Val Val Val Leu Tyr Cys 195 200 205 Lys Val Gly Gln Asp Leu Gly Phe Pro Gly Ala Trp Arg Phe Gln Val 210 215 220 Ser Leu Glu Leu Pro Asp Pro His Pro Cys Leu Ser Val Cys Gln Gly 225 230 235 240 Asn Lys Gly Lys Leu Gly Val Ile Val Ser Ala Tyr Met His Tyr Ser 245 250 255Lys Ile Ser Ala Gly 260 [0203]

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gag ctg agg agt cga ctg gcc act ctg gag aca gaa 607 His Val Glu Ala Glu Leu Arg Ser Arg Leu Ala Thr Leu Glu Thr Glu135 140 145 150gca gcc cag cac caa gct gtg gtt gac ggt ctc acc cgg aag tac atg 655 Ala Ala Gln His Gln Ala Val Val Asp Gly Leu Thr Arg Lys Tyr Met 155 160 165 gaa acc att gag aag ctg cag aac gac aag gct aaa cta gaa gtg aaa 703 Glu Thr Ile Glu Lys Leu Gln Asn Asp Lys Ala Lys Leu Glu Val Lys 170 175 180 tct cag-act-cta-gaa-aag gaa-gcc-aag-gaa-tgt cga ctt cga acg gaa 751 Ser Gln Thr Leu Glu Lys Glu Ala-Lys-Glu-Cys-Arg-Leu-Arg-Thr-Glu 185 190 195 gaa tgt caa tta cag-tta-aag-act-ctt cat gaa gat ttg tca ggt aga 799 Glu Cys Gln Leu Gln Leu Lys Thr Leu His Glu Asp Leu Ser Gly Arg 200 205 210 tta gag gaa tcc tta tca atcatc aat gaa aaa gta cct ttt aat gat 847 Leu Glu Glu Ser Leu Ser Ile Ile Asn Glu Lys Val Pro Phe Asn Asp215 220 225 230aca aaa tat agt cag tac aac gctctgaac gtt cca ctc cac aat agg 895 ThrLys Tyr Ser Gln Tyr Asn Ala Leu Asn Val Pro Leu His Asn Arg 235 240 245aga cac 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cca gta aga actaaa aaa cgt ttc aca 147 Thr Gln Ser Ala Ala IleVal Pro Val Arg Thr Lys Lys Arg Phe Thr 30 35 40 cct cct att tat caa cct aaa ttt aaa aca gaa aag gag ttt atgcaa 195 Pro Pro Ile Tyr Gln Pro Lys Phe Lys Thr Glu Lys Glu Phe Met Gln 45 50 55 cat gcc cgg aaa gca gga ttg gtt att cct cca gaa aaa tcg gac cgt 243 His Ala Arg LysAla Gly Leu Val Ile Pro Pro Glu Lys Ser Asp Arg 60 65 70 tcc ata cat ctg gcc tgt aca gct ggt ata ttt gat gcc tat gtt cct 291 Ser Ile His Leu AlaCys Thr Ala Gly Ile Phe Asp Ala Tyr Val Pro 75 80 85 90cct gag ggtgat gca cgc ata tca tct ctt tca aag gag gga ctg ata 339 Pro Glu Gly Asp Ala Arg Ile Ser Ser Leu Ser Lys Glu Gly Leu Ile 95 100 105 gag aga act gaacga atg aag aag act atg gca tca caa gtg tca atc 387 Glu ArgThr Glu Arg Met Lys Lys Thr Met Ala Ser Gln Val Ser Ile 110 115 120 cgg agg ata aaa gactat gat gcc aac tttaaaata aag gac ttc cct 435 Arg Arg Ile Lys Asp Tyr Asp Ala Asn Phe Lys Ile Lys Asp Phe Pro 125 130 135 gga aaa gctaag gat atc tttatt gaa gct cac ctt tgt cta aat aac 483 Gly Lys Ala Lys Asp Ile Phe Ile Glu Ala His Leu Cys Leu Asn Asn 140 145 150 tca gac cat gac cgactt cat accttg gta act gaa cac tgt ttt cca 531 Ser Asp His Asp Arg LeuHis Thr Leu Val Thr Glu His Cys Phe Pro155 160 165 170gac atg act tgg gac atc aaa tat aag acc gtc cgc tgg agc ttt gtg 579 AspMet Thr Trp Asp Ile Lys Tyr Lys Thr Val Arg Trp Ser Phe Val 175 180 185 gaa tct tta gag ccc tct cat gtt gtt caa gtt cgc tgt tca ag t atg 627 Glu Ser Leu Glu Pro Ser His Val Val Gln Val Arg Cys Ser Ser Met 190 195 200atg aac cag g gc aac gtg tac ggc cag-atc-acc-gta-cgc atg cac acc 675 Met-Asn-Gln-Gly-Asn Val Tyr Gly Gln Ile-Thr-Val-Arg-Met-His-Thr 205 210 215 cgg cag act ctg gcc-atc-tat-gac-cgg ttt ggc cgg ttg atg tat gga 723 Arg GlnThr Leu Ala Ile Tyr Asp Arg Phe Gly Arg Leu Met Tyr Gly 220 225 230 cag gaa gat gta ccc aag gat gtc ctg gag tat gtt gta ttc gaa aag 771 Gln Glu Asp Val Pro Lys Asp Val Leu Glu Tyr Val Val Phe Glu Lys235 240 245 250cag ttg aca aac ccc tat gga agc tgg aga atg cat acc aag atc gtt 819 Gln Leu Thr Asn Pro Tyr Gly Ser Trp Arg Met His Thr Lys Ile Val 255 260 265 ccc cca tgg gca ccc cct aag cag ccc atc ctt aag acg gtg atg atc 867 Pro Pro Trp Ala Pro Pro Lys Gln Pro Ile Leu Lys Thr Val Met Ile 270 275 280 cct ggc cct cag ctgaaa cca gaa gaa gaa tat gaa gag gca caa gga 915 Pro GlyPro Gln Leu Lys Pro Glu Glu Glu Tyr Glu Glu Ala Gln Gly 285 290 295 gag gcc cag aag cct cag cta gcc tgatgacaaaaatgacttct agggtgaagc 969 Glu Ala Gln Lys Pro Gln Leu Ala 300 305 ctgggtgatg aggctgctgg aagctttgaa gtctcccatt cccctcatgc tataaaaaga 1029 actacctttg ttctctccca tcctgctcag gtcttttcag cagtctcatc atcagcaacc 1089 atgactgatg actgggccct agcaggtggc aggtataaca tggccatgga cactcttctt 1149ttttaaattttatgtctag(s)c ttctgagtct agatgaaaga cagtatgttt cagagaacat 1209 tggatatcag tttttcccac agcagggact gtgagagaca accagcagca tcctctttgt 1269 aatcacaggg cagggatcag agtttgaaat gaaatgttgt cagggtgttg gaaaaatttt 1329 ggtgagttct gcacatttcc cctggttcag gctgggcatg gaccagcctt cagatggcag 1389 aagtggaaga tgagcctact tgtgagcgat gtgactttaa ggaaatgagg actggggaag 1449 aataattagt gtttataaga catttaagag gccctttttc atatactgac tcactgatga 1509 atcagcattt gcattttatg gaaaaatata aatgcaaaga aataatttaa aaaaaaaaaa 1569aaaaa 1574 [0207]
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* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the result of investigating the amount of manifestations of the COL03279 imprint object in 35 sorts of human tissues (organ) using ** and the PCR method.
[Drawing 2] It is the result of investigating the amount of manifestations of the COL06772 imprint object in 35 sorts of human tissues (organ) using ** and the PCR method.
[Drawing 3] It is the result of investigating the amount of manifestations of the ADKA01604 imprint object in 35 sorts of human tissues (organ) using ** and the PCR method.
[Drawing 4] It is the result of investigating the amount of manifestations of the ADSU00701 imprint object in 35 sorts of human tissues (organ) using ** and the PCR method.
[Description of Notations]
The figure of a publication in a complete diagram and the alphabet are as follows.
A suprarenal gland, 02:brain, 03:caudate nucleus, 04:hippocampus, 05:substantia nigra, 06 : 01: A thalamus, 07 : The kidney, 08:pancreas, 09 hypophyses, 10:small intestine, 11:bone marrow, 12 : An amygdala, 13:cerebellum, 14:corpus callosum, 15:embryo brain, 16:embryo kidney, 17: Embryo liver, 18:embryo lungs, 19:heart, 20:liver, 21 : Lungs, 22: -- lymph gland and 23: -- a mammary gland, 24:placenta, 25:prostate gland, 26:salivary glands, 27:skeletal muscle, and 28: -- a spine, 29:spleen, 30:stomach, 31:testis, 32:thymus gland, and 33: -- the thyroid, 34:trachea, 35:uterus, Pr:plasmid, and M:molecular weight marker

[Translation done.]